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THE PATHOLOGY OF EXPERIMENTAL YELLOW FEVER IN THE *MACACUS RHESUS* *

I. GROSS PATHOLOGY

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INTRODUCTION

This report is based on the necropsy findings in sixty-eight *Macacus rhesus* monkeys fatally inoculated with the strain of yellow fever virus obtained from Asibi, an African native. A brief clinical history of this patient has been presented in an earlier report,[1] where also the gross pathology of a few individual animals was described and a brief summary of the general findings given. This and other articles to follow on the pathology in the *M. rhesus* are intended to amplify the earlier report.

At the time of writing, Jan. 1, 1928, eighty-eight animals of this species were inoculated in various experiments connected with the study of the virus. Eleven did not succumb; six of these were protected with human convalescent yellow fever serum, one was injected with washed red blood cells, one was bitten by apparently non-infective mosquitoes, and three were evidently insusceptible. Of the seventy-seven monkeys that died, nine fatalities were obviously not due to experimental infection, but are to be accounted for as follows: two were killed on the first day of fever, two died of pneumonia, two of generalized tuberculosis, two of dysentery and one of acute sepsis. This analysis reveals that sixty-eight animals were experimentally infected and available for this study.

* The studies and observations on which this paper is based were conducted with the support and under the auspices of the International Health Division of the Rockefeller Foundation.

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The transmission of the virus was accomplished in various ways. One rhesus monkey died after inoculation with the patient's blood; twenty-five after inoculation with infectious monkey blood; two with serum and five with serum filtrate, monkey to monkey transfer; twenty-six after being bitten with infective *Aedes* (*Stegomyia*) *aegypti;* and nine following miscellaneous experiments, such as injection of mosquito emulsions and of blood stored for various periods of time.

For the purpose of a control, two normal rhesus monkeys in excellent condition were killed and studied. In addition, numerous other uninoculated monkeys of this species dying of natural causes were examined. These causes of death may be classified as dysentery, tuberculosis, pneumonia and undetermined; at no time, however, did the prevalence or incidence of any one disease suggest the existence of an epidemic. No gross lesion was found in these animals similar to yellow fever pathology, except in one or two animals dying from dysentery, in which a few petechial hemorrhages were seen in the gastric mucosa.

The experimental animals were apparently full-grown adults, although their ages were undeterminable. In this series, males were more numerous than females. Their lengths over head and trunk varied from 35 to 50 cm., averaging about 40 cm., and their weights were from 1600 to 3000 gm. Animals obviously sick were avoided in experimentation as much as it was possible, and as a rule monkeys coming to postmortem examination were fairly well to very well nourished.

Diseases of recognizable pathology concurrent with the experimental infection were found in a relatively few animals. One monkey (*M. rhesus* No. 253 A) had tuberculosis of the lungs, while one (No. 334) had generalized tuberculosis; a mild bronchopneumonia was present in one (No. 384); another animal (No. 349) showed a localized peritonitis; and a sixth rhesus (No. 229) had evidence of an acute pancreatitis. Microscopic examination of these animals has confirmed the existence of the complicating gross lesions, together with a yellow fever infection, except in the case of No. 349 which has not been studied microscopically. In the case of No. 229, it cannot be proved whether yellow fever or the acute pancreatitis caused death, but ample gross and microscopic evidence is manifest to indicate the presence of a severe yellow fever infection.

Postmortem Observations

Jaundice: Jaundice appeared as a light yellow to lemon yellow color with sometimes a tendency to a greenish yellow shade, especially in the case of body fluids. The color was not brownish or like that seen in obstructive jaundice in man.

A yellowish tint of the skin was best seen in the upper part of the face and eyelids but, in this group of animals, discoloration of these parts was irregular and mild. It was most evident in those animals that were examined some time postmortem.

Jaundice of the tarsal conjunctivae was present distinctly or markedly in thirty and was mild in an additional seventeen of the sixty-eight monkeys. Just antemortem, capillary congestion of the conjunctivae often prevented the determination of jaundice which became evident after death with the emptying of the vessels. The subcutaneous tissue and fat were definitely icteric in thirty animals, while the larynx was regularly colored light yellow to deep lemon yellow. Jaundice was noted as distinct in the trachea of twelve and as mild in an additional eighteen. Icteric omental fat and gastric mucosa were seen five times each and this characteristic was noted in the external surface of the stomach fourteen times. Commonly the cleaned mucosal surface of the intestines was pale and yellowish in the absence of congestion or hemorrhages.

Often the peritoneal fluid was increased sufficiently to note its color, which was always yellow or greenish yellow. The pericardial fluid had, as a rule, the same abnormal coloration. The urine, when present, was regularly greenish yellow, which often was markedly intense.

Two parts almost invariably jaundiced were the larynx and the surface of the large vessels extending from the heart. In the case of the aorta, fifty-six were noted as being definitely to markedly jaundiced, seven were mildly jaundiced, while only two were negative and three are unrecorded. Here the color varied from a mild yellow tint to a deep lemon yellow and was most intense at the base of the aorta.

Jaundice of most parts was recognizable, although confusion was possible with the normal color of fat. The tarsal conjunctivae, aorta, trachea and larynx were dull or pearly white in the normal rhesus and in animals dying of natural causes. Peritoneal and pericardial

fluids and urine were colorless in control animals, except occasionally when the urine of sick, non-experimental monkeys was yellowish.

The marked irregularity in occurrence and degree of icterus is to be noted. The more intense jaundice was usually found in animals that either ran a long clinical course or were examined several hours postmortem; this relation, however, was not constant. There was no regularity in the parts involved except in the case of the larynx, aorta and other large vessels near the heart.

Pallor: This characteristic was commonly seen in the buccal, gastric and intestinal mucosa. Certain organs were likewise pale, notably the liver, kidneys and, to a certain degree, the heart.

Hemorrhage: The tendency to hemorrhage was manifest in various ways. Blood was definitely found at the base of several teeth, usually the upper incisors, in eleven animals, and in two additional monkeys the amount was so small as to make the finding uncertain. These eleven cases of positive bleeding gums were found in the absence of pyorrhea or evidence of trauma, and are exclusive of those caused to bleed antemortem by gently rubbing the lip over the gum. The gums just antemortem were often soft and purplish, and bleeding was induced in seven of twelve animals so examined. Twenty-two uninoculated monkeys were similarly treated and after prolonged and vigorous rubbing of the lips over the gums, blood appeared oozing from the margin of the gums in two animals showing gross evidence of pyorrhea.

Petechial hemorrhages occurred on the pleural surface of the lungs in about two-thirds of these experimental monkeys (Fig. 1). Somewhat fewer specimens displayed hemorrhages in the lung tissue, although it was not practical to expose much tissue by sectioning. The hemorrhages appeared as bright red dots 2 to 4 mm. in diameter and in number were from a half to two dozen on the surface of both lungs, usually closer to the upper figure.

It was very unusual to find any tendency to hemorrhage in the heart, and this was never observed in the musculature or epicardium. Twice, however, irregular diffuse hemorrhages were present in the endocardium.

The stomach in twenty-six, or a little over a third of these monkeys, contained variable quantities of altered blood (Fig. 2). The blood appeared as fine black or chocolate-colored threads and flecks, and when present in large amounts, blackened the whole stomach

contents. Usually the streaks of blood could best be seen in the mucus next the mucosa. Sometimes fresh red blood was found streaking from the mucosa, but in no instance could any gross lesion be demonstrated in the mucosal surface. Hemorrhage and congestion of the gastric mucous membrane were present seventeen times and usually not in the same animal. These characteristics were variable in degree, and the scattered and minute petechiae were largely in the fundus while the congestion occurred principally near the cardia. Altered blood in the stomach contents was generally independent of hemorrhage and congestion, and conversely, the latter two findings were not commonly concurrent with the black vomitus.

The lack of association of blood in the contents with hemorrhage or congestion of the mucosa, was again demonstrated in the small intestine. In twelve instances, mucosal changes were evident, while altered or fresh blood was found in thirteen specimens. These findings were present more often in the ileum than in the duodenum or jejunum, although occasionally unequally distributed throughout the small intestine. The quantity of altered blood varied from a definite smearing of the mucosal surface with partially altered blood to large masses of brownish red or chocolate-colored contents. No break in the continuity of the mucosa and no evidence of intestinal infection were found in these cases. Although sometimes present in the same animal, there was no definite relation in the occurrence of the hemorrhages or blood content of the stomach to those of the small intestine.

No hemorrhages were seen in the skin, peritoneum, retroperitoneal or perinephritic tissues, kidneys, liver, bladder mucosa, lymph nodes, brain or cerebral meninges.*

In the control animals, the tendency to hemorrhage or accumulation of altered blood in the gastro-intestinal tract was not observed except in two monkeys having manifestations of a dysenteric infection, in which petechial hemorrhages were present in the gastric mucosa.

Lungs: The lungs were always soft, pink and air-filled and the petechial hemorrhages were conspicuous against the pale background. Practically every specimen showed parasitic cysts of *Pneu-*

* Although in this series no hemorrhages were found in the adrenal glands in gross, they occurred in this organ in two monkeys inoculated with another strain of this virus.

*monyssus griffithi.** These cysts were 3 to 5 mm. in diameter and had a soft yellowish center; they lay just under the visceral pleura and less commonly within the lung tissue. Microscopically, these areas contain a cyst with a definite wall and are probably connected with a bronchiole since some such cysts are lined by a layer of epithelial cells. The wall is densely infiltrated with endothelial leucocytes, lymphocytes, polymorphonuclears and a few eosinophiles. Cross-sections of a mite are sometimes observed in the cysts. In gross, narrow bands of hemorrhage often surrounded the cysts in the experimental monkeys, while in control animals these areas were almost colorless except for the yellowish center.

Heart: The weight of this organ varied with the size of the animal. Jaundice of the lining of the large vessels leading from the heart has been discussed. In addition, numerous specimens had a yellowish tint of the endocardium, auricles and valvular rings. Pallor of the musculature in some instances was conspicuous.

Liver: The weight of the liver varied somewhat but was roughly in proportion to the size of the monkey. There was no relation between the weight and degree of infection.

The color of this organ was the most conspicuous feature. When the abdomen was opened immediately after death, the liver was pale, pinkish yellow which faded with exsanguination to a markedly pale, yellowish brown or cream color (Fig. 3). If the animal had been dead a short time when examined, the liver was commonly very pale and cream-colored, with the surface sometimes splotched with red that blanched on cutting the large vessels and on handling. On section, the liver tissue usually had the same color as externally. This constant color characteristic gave the liver the so-called "boxwood" appearance, and indeed, the color generally matched a boxwood ruler at hand. The pallor and color of the liver were in sharp contrast to the deep red-brown liver of control animals.

Mottling of the surface with fine lobular markings was observed in most specimens, although often the liver surface was of a uniform pale cream color, especially on section. These lobular markings were best seen with a hand lens on the external surface; as a rule, a fine

* Mrs. Sophia Connal of the British Medical Research Institute, Yaba, very kindly identified these parasites as being *Pneumonyssus griffithi*, Order Acarina: mites. They correspond in every way to the parasite described by Gay and Branch[3] occurring in the *Macacus rhesus* and called by them *Pneumonyssus foxi.*

red dot representing the central vein, surrounded by a wide yellowish zone and a red interlacing periphery could be made out. Hemorrhagic areas were not demonstrable externally or within the liver tissue.

The surface of the liver was smooth and regular and the edges sharp. By external examination, it was about as firm as normally, but on section, the tissue was often soft and friable, and sometimes cheesey in consistence. The surface of the sectioned tissue was smooth and glassy, reflected light and generally appeared fatty. There was an obvious diminution in the amount of blood in the liver, and on sectioning, the tissue was dry and almost bloodless.

The gall-bladder was not remarkable. All specimens contained 3 or 4 cc. of more or less thick, mucoid, dark green bile. No resistance was offered to the flow of bile on pressing the gall-bladder and the intestinal contents were always bile-stained.

Spleen: The weight of the spleen varied considerably, but on the whole there was an increase over the weight of this organ in the control animals. The spleen was firm, its edges generally rounded and its surface smooth and normally colored. On section, this organ was dark red and firm, and blood did not ooze freely from the surface. Normally, the malpighian corpuscles are large, gray, prominent and closely dotting the splenic tissue. In the experimental monkeys, these bodies were regularly reduced in size, sometimes being barely visible, and separated by red pulp.

Kidneys: These organs were increased in weight over those of the control monkeys. Externally they were firmer than normal and, while encapsulated, somewhat pale and bluish red; often there was also a definite brownish tinge externally. The capsule stripped readily from a smooth pale brown surface. On section, the cortex and pyramids were always pale. The cortex was either light reddish brown or yellow-brown and the medulla was usually of the same color, although occasionally the pyramids were redder than the cortex. Sometimes the cortical capillaries were traced, but as in the liver, very little blood oozed from the tissue. A constant characteristic was the presence of fine, dull gray cortical rays easily seen with the naked eye and contrasted to the surrounding pale brown tissue. The cortex was firm and appeared full and tense. The glomeruli could not be made out. The pelvis and ureters were not remarkable.

Adrenal Glands: These structures were not affected in gross in this group of experimental animals aside from occasional congestion.

Pancreas: The pancreas was normal, with the exception of the one case (No. 229) mentioned above, in which there was an acute pancreatitis of unknown etiology.

Gastro-Intestinal Tract: The esophagus was not remarkable except for an occasional smearing with vomitus and frequent mild jaundice of a pale mucosa. The pathology in the stomach and small intestine has been discussed in connection with the subjects of hemorrhage, jaundice and pallor. The large intestine deserves mention because of the frequent occurrence of streaks of bright red blood on the surfaces of the mucosa and feces. No significance as regards experimentation is attached to this finding because of the occasional presence of round worms both free in the intestinal lumen and embedded in the mucosa. Worms were also extracted from submucous and, very rarely, subserous cysts filled with purplish blood. These worms on examination agreed with the description recently given by Branch and Gay [3] of worms found in the *M. rhesus* and which they identified as being *Oesophagostomum brumpti*. The parasites were not constantly associated with diarrhea and their pathogenicity is yet to be determined.

Lymph Nodes: Axillary and inguinal nodes were occasionally congested and slightly enlarged, but as a rule these structures were small and pale. Mesenteric lymph nodes varied in size and were pale, yellowish pink. Peribronchial and peritracheal nodes were normal except in the rare instances of pulmonary tuberculosis, No hemorrhagic nodes have been seen.

Urinary Bladder: The bladder mucosa often appeared yellowish, and hemorrhage or congestion was never observed. The urine was highly colored and either yellow or greenish yellow. It regularly gave positive tests for albumin and bile and often showed hyaline and granular casts.

Genitalia: Jaundice of the vaginal mucosa was the only significant change found in the female genital tract. Similarly, the prepuce of males was generally icteric; otherwise, the male genitals were normal.

Brain: The brain was removed and weighed in several animals. It appeared normal externally and on section, and the weight varied directly with the size of the monkey. The cerebrospinal fluid was tinged with yellow in a few instances.

Other Parts: The organs of the neck, submaxillary glands, voluntary muscle and costal cartilages were not remarkable. The tongue was pale but otherwise normal. In instances of extreme jaundice, the tendons of large muscles and bursal surfaces were distinctly yellowish.

Weights of Organs

The liver, heart, kidney and spleen were routinely weighed. Weights of organs from sixty-three monkeys are available for analysis and the length over head and trunk is taken for the basis of comparison. When all experimental monkeys are compared with the

Table I

Weights of Organs

	Number of animals	Length over head and trunk	Liver	Heart	Kidney	Spleen
		cm.	gm.	gm.	gm.	gm.
Average in normal monkeys (killed)	2	43	84	16.5	13	4.5
Average in monkeys dying of natural causes	17	40.3	77.7	10	14	4
Average in all non-experimental monkeys	19	40.6	78.4	10.6	13.8	4
Average in experimental monkeys	63	40.6	83.2	11.7	17.5	5.5

normal animals, the average weight of the kidneys is 42 per cent and that of the spleen is 22 per cent over the corresponding weights in the normal, even though the average length of the experimental animals was less than that of the normals. The comparison of weights of organs with only two animals is obviously open to error. Another available method of determining any variation in organ weights of experimental animals, is to compare their weights with those of seventeen rhesus monkeys dying from miscellaneous, non-experimental causes. When this is done, we can compare animals of the same average length and find that the kidneys are 27 per cent and the spleen 37 per cent heavier in experimental monkeys than in the other group. The heart and liver are practically the same in the two groups, although slightly heavier in the experimental series.

Obviously, under the circumstances, no mathematical statement of the increase in weight of the kidneys and spleen of experimental monkeys can be made as a group, but many individual animals illustrated the point that in the animals dying of yellow fever, the weight of these organs is generally increased. A few examples are included in the accompanying tables which summarize the analysis of organ weights.

TABLE II

Weights of Organs of Selected Individual Monkeys

Animal No.	Length over head and trunk	Liver	Heart	Kidney	Spleen
	cm.	*gm.*	*gm.*	*gm.*	*gm.*
312	42	74	13	20	7
316	39	92	8	18	7
325	42	89	16	18	8
339	48	140	19	31	9
342	36	65	10	17	7
360	42	100	14	20	7
363	43	73	13	19	7
391	37	64	10	15	6
Average	41	87	13	19	7

SUMMARY

The gross pathology in sixty-eight *Macacus rhesus* monkeys fatally inoculated with the Asibi strain of yellow fever virus is described. The variety of routes and modes of infection furnished ample opportunity to study the effect of the method of transmission of virus on the pathology produced. As yet, however, no variation in the gross findings has been observed attributable to the method of infecting the animal, and all variations noted are within the limits found to obtain in a large number of animals infected by any one method, *e. g.*, intraperitoneal injection of blood.

The principal gross lesions were jaundice, hemorrhage and pallor of various parts, and the changes in the liver, kidney and spleen. Jaundice and hemorrhage were variable in occurrence and degree, but the pallor of the liver and kidney and the buff or yellowish "boxwood" color of the liver were constant and definite. The surface of sectioned liver tissue appeared glassy and fatty and the tissue

was generally friable, almost bloodless, and obviously necrotic. The spleen and kidneys were commonly increased in weight over those of control animals. This seemed to be due in the case of the spleen to congestion and in the kidneys to an apparent acute degenerative change.

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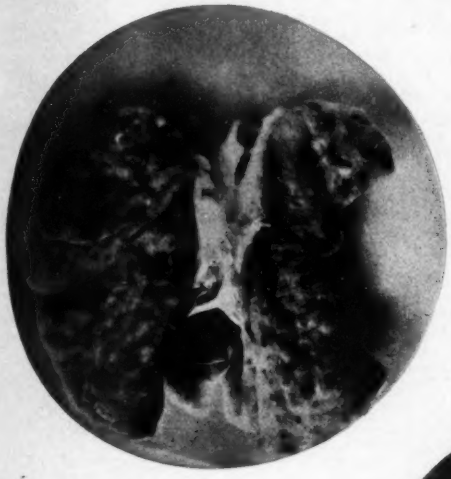
DESCRIPTION OF PLATE

PLATE 93

FIG. 1. *M. rhesus* No. 338. Petechial hemorrhages of the lungs. This specimen shows an unusually large number of hemorrhages. It also demonstrates about a half dozen cysts of *Pneumonyssus griffithi* each surrounded by a zone of hemorrhage; the cyst at the apex of the right lung is best illustrated. Three-fourths natural size.

FIG. 2. *M. rhesus* Nos. 316 and 368. Two opened and partially everted stomachs, showing altered blood ("black vomitus") covering the mucosal surface. Three-fourths natural size.

FIG. 3. *M. rhesus* No. 411. Contrast of pallor of liver of experimental monkey (left) with deeply colored liver of uninoculated monkey (right). Note that the difference in size in organs was due to the difference in size of the animals, the experimental monkey being 38 cm. over head and trunk, while the other, dying of dysentery, was 44 cm. Both specimens removed at the same time, were washed in water, immersed in Kaiserling fluid and photographed at once. About one-third natural size.



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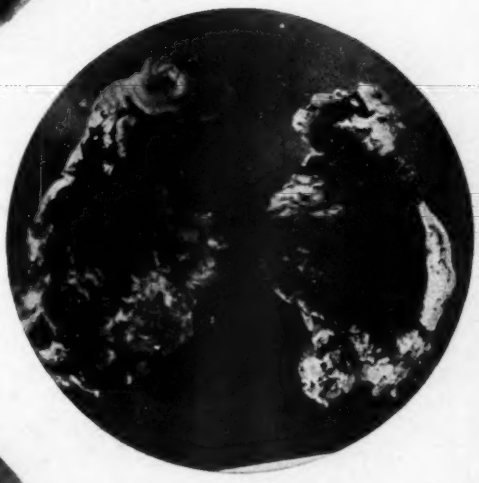

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Hudson

Pathology of Experimental Yellow Fever in *Macacus Rhesus*, I

THE PATHOLOGY OF EXPERIMENTAL YELLOW FEVER IN THE *MACACUS RHESUS* *

II. MICROSCOPIC PATHOLOGY

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INTRODUCTION

This report on the histopathology of experimental yellow fever is based on the microscopic study of tissues of thirty *Macacus rhesus* monkeys fatally inoculated with the Asibi strain of the virus.[1] All these animals are included in the report on the gross pathology[2] found in the sixty-eight monkeys of the same species.

The virus was transmitted in various ways to the animals microscopically studied. These methods may be grouped in the following manner:

Blood from patient (Asibi)	1
Blood and organ emulsion from monkey	1
Blood from monkey	8
Blood from monkey applied to scarified skin	1
Monkey serum filtrate (Berkefeld N)	1
Monkey serum filtrate (Seitz asbestos)	1
Mosquito (*Aedes aegypti*) transmission	16
Emulsion of mosquitoes (*A. aegypti*)	1

Monkeys were examined and tissues preserved as soon after death as possible, because of rapid postmortem changes. The necropsy was done at once in the case of twenty animals, within one hour after the death of seven, and a few hours postmortem in three. In a few instances, chloroform was used to hasten the end of moribund animals in order to make the postmortem examination by daylight and, compared to the large number of animals not so treated, the small amount of chloroform needed did not seem to alter the microscopic pathology.

The diet of all monkeys has been rice, oranges, bananas, bread, evaporated milk and water. For purposes of comparison, the tissues of two apparently normal and several rhesus monkeys dying from natural causes (dysentery, tuberculosis and undetermined) were available.

* The studies and observations on which this paper is based were conducted with the support and under the auspices of the International Health Division of the Rockefeller Foundation.

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The tissue fixatives employed were 10 per cent formalin and Zenker's fluid and the staining method was routinely hematoxylin and eosin; occasionally van Gieson and Giemsa preparations were studied. For the demonstration of fat, frozen sections were stained with scarlet red and counterstained with hematoxylin.

Pathologic Histology

Liver: This organ furnishes the most extensive and constant pathology and shows chiefly fatty degeneration and necrosis.

No fat is found in one normal rhesus and in the other, it occurs as large droplets sparsely sprinkled through the lobule. In monkeys dying from natural causes, there is only a small amount of periportal fat. In paraffin sections of the experimental tissues, large and small vacuoles are evident, especially in the normal or moderately altered liver cells, such as in the periportal region. In frozen sections, fat is demonstrated in every liver. In the majority of specimens, it is present in extreme amounts, occurring as minute to large droplets in practically every parenchymatous cell. In numerous instances, more fat, usually as larger droplets, is found in the periportal and central zones of the lobule. In one liver (No. 304) in which the necrosis is mild, fatty degeneration is limited to the midzone.

In the normal liver, the parenchymatous cells are regular, polyhedral and arranged roughly in columns; the neutrophilic cytoplasm is not uniformly stained and gives a positive test for glycogen (Best's carmine stain). The cytoplasm of the liver of monkeys dying from natural causes is smoothly stained and neutrophilic. In contrast to these control animals, the hepatic cells of experimental monkeys are not arranged in columns in the affected regions, but are jumbled, rounded and irregular in size and shape. No glycogen is found in these sections (one monkey tested).

Early degenerative changes are represented by the loss of the normal cell relation and the presence of mild to marked eosinophilic-staining properties. More advanced stages of necrosis appear as acidophilic, coarsely granular cytoplasm in distorted cells. Finally, disintegration takes place and the cytoplasm is either intensely or poorly stained, and granular; the cell wall may be entirely indefinable. In about two-thirds of the livers, hyaline degeneration is one of the forms of cytoplasmic change. Hyalin is seen as small, irregular

homogeneous, well-stained masses within the cell and occurs in only a relatively few cells of the section.

Nuclear changes coincide with the changes of the cytoplasm. Large, poorly stained, vesicular nuclei with prominent nucleoli are found in the necrobiotic liver cells. In such nuclei, minute, dull red granules are often found. They were present in half the thirty specimens studied; in an additional six specimens, this type of nuclear change is less often present. Occurring in half the livers of this group, variable numbers of nuclei appear as small, round, intensely acidophilic dots. This form of degeneration is usually seen in the comparatively rare, small liver cell having smooth, intensely stained cytoplasm. The nucleus finally disappears in advanced necrotic cells either by lysis or karyorrhexis. In eleven specimens, karyorrhexis is common or marked, while in five others it occurs relatively infrequently. When karyorrhexis is marked, scattered nuclear débris is conspicuous in the hepatic cells throughout the section. Mitosis is not found.

The degree of necrosis varies only slightly among the specimens and in only one instance (No. 304) is it limited to the early stages of degeneration. No effort has been made to determine the variation in degree of necrosis throughout the liver, but there is reason to believe that such exists. The variation in any one section, however, is negligible, all lobules being equally affected.

The extent of necrosis and necrobiosis in the lobule, on the other hand, is not constant among the specimens except that the midzone is always attacked. In two cases, the midzone alone is necrotic, mild in one (No. 304) and extreme in the other (No. 312). In one-third of the livers a wide intermediate zone encroaching upon the periportal and central zones is involved; in an additional third, parenchymatous cells of all zones are degenerated, leaving only a fringe of intact but vacuolated cells about the portal region and central vein; and in the other instances, a border of periportal cells alone is undegenerated. In the majority of cases of extensive necrosis, the most extreme changes are midzonal. Under such circumstances, the most numerous necrotic cells are in the intermediate zone, while toward the limiting structures the necrotic cells, mingled with the necrobiotic cells, decrease and the latter type increase in numbers. The same is true of necrobiotic cells in relation to the intact and normal cells. In no case is either central or periportal necrosis alone encountered.

In spite of disintegration of parenchymatous cells, no collapse of cell space is evident and lobules retain their normal shape and size.

Sinusoids are easily followed except when distorted in extremely necrotic areas. Their lining cells are rarely enlarged and only occasionally phagocytic. The nuclei of these cells are well stained and sometimes comprise the only intact nuclei of a necrotic region. Congestion is not a conspicuous feature and when present is irregular and mild. No thromboses are evident. Hemorrhage is rare and is found to an appreciable degree in one instance in the midzone and in another about the central vein. On the other hand, a few to numerous scattered red blood cells are seen to be extravascular in one-third of the specimens.

Portal areas are commonly not remarkable aside from mild and variable lymphocytic infiltration. Often endothelial leucocytes and occasionally polymorphonuclears are found in these regions and in one instance necrosis is obvious. There is no fibrosis.

In this organ, inflammatory cells are generally present in the necrotic regions but their presence and numbers do not depend on the degree or extent of parenchymatous necrosis. In twenty-four specimens, scattered polymorphonuclear leucocytes are in the sinusoids, and in three of these, they occur in foci as well; in half of these twenty-four instances, these cells are also among or in necrotic liver cells, but not in large numbers. In about the same number of animals, not always, however, in the same specimens, endothelial leucocytes are found, both intravascular and extravascular. In the latter situation, they are occasionally large and phagocytic, sometimes containing acidophilic granular débris. While inflammatory cells commonly accompany the necrosis of parenchymatous cells, their occasional complete absence indicates that they do not constitute an essential part of the pathology. Lymphocytes and eosinophiles are not found.

Kidney: Fatty degeneration is not demonstrable in this organ of the normal and uninoculated monkeys. In paraffin sections of numerous experimental tissues, fine vacuoles are seen near the base of epithelial cells of cortical tubules. In frozen sections stained for fat, large amounts of this substance are demonstrated as minute to small droplets in the tubular epithelium of twenty-seven of the thirty specimens. The convoluted tubules show the most advanced fatty degeneration and when less than extreme amounts of fat are present,

the straight tubules are spared. Fat is not stained in the glomerular structures or in parts other than the tubular epithelium.

In non-experimental animals, the renal tubular margin is slightly irregular, but the lumen is distinctly patent. In experimental sections, the epithelial cells of convoluted tubules, more than of straight tubules, are swollen, slightly granular and occasionally acidophilic. In twenty-three specimens, this acute degenerative change is marked almost to the occlusion of the lumen of the convoluted tubules; it is mild in three others and inconspicuous in three. Necrosis is not extensive in these monkeys; however, a relatively few to numerous necrotic epithelial cells of cortical convoluted tubules are evident in the majority of cases. Such cells are granular, disintegrated and without nuclei, and in the presence of one or two necrotic cells in the cross-section of a tubule, the margin is ragged.

The nuclei of these cells showing cloudy swelling are usually well preserved, although in a few instances some are poorly stained. Ten specimens demonstrate pyknotic nuclei of epithelial cells, which are more generally found in straight than in convoluted tubules. Karyorrhexis is relatively uncommon and is conspicuous in only two instances.

No inflammatory cells are found in kidney sections, either in degenerating cells or in the tubules. Congestion is not marked and when present, occurs mildly and irregularly. There is no indication of hemorrhage and no red blood cells lie in the tubules.

Tubules of the cortex and medulla as a rule contain varying amounts of granular or circular débris. Few to many granular and hyaline casts are in straight and collecting tubules of about one-half the specimens. The two varieties are not always in the same animal, although as a rule they are coincident. Sometimes they are distinctly bile-stained.

In six animals, deposits of hematoxylin-stained material are seen in collecting tubules and less frequently in convoluted tubules. They are best demonstrated in formalin-fixed tissues as either clumps of minute granules, larger solid masses, or discs. Their color varies from slate-blue to almost black. In frozen sections counterstained with hematoxylin, they are conspicuously darkly stained. After treating paraffin sections with silver nitrate according to Klotz' method, these deposits appear dark brown or black. Immersion in a weak solution of nitric acid before staining makes it difficult to

demonstrate the deposits with hematoxylin; those found are only faintly colored, whereas cell nuclei are not altered in their staining properties. With these findings and the demonstration of form, location and hematoxylin-staining, it seems that such casts are calcareous deposits. At least one source of this variety of cast may be a form of degeneration of the epithelium of collecting tubules, since one can trace similar intracellular masses through the changes of the cell to its necrosis and disintegration. Sometimes these calcareous deposits form a rough ring about the lumen of the collecting tubules, either within or on the surface of necrotic cells or in the entire absence of epithelium.

Glomerular capillaries are sometimes congested and their spaces occasionally contain a small amount of granular débris; otherwise these structures are not remarkable. The renal capsule and pelvic mucosa are unaltered.

Heart: In comparison to the normal controls, paraffin sections show little change in cellular structure. In a few specimens, the fibers are irregularly stained, the cross-striations are indistinct and the longitudinal fibrils are prominent. Inflammatory cells are not present in any part. Congestion of small vessels is noteworthy in six cases and mild in two others. Diffuse recent hemorrhage without reacting inflammatory cells is found under the endocardium and among the neighboring muscle fibers in one animal.

In frozen sections stained for fat, it is demonstrated as very minute droplets, usually distributed unequally among the fibers. In six specimens the amount is extreme, nearly all the fibers being involved; in sixteen the fatty degeneration is marked; in five it is mild; no fat is found in two; while in one case the tissue is not available for study.

Spleen: Sections of normal rhesus spleens show much lymphoid tissue with active germinal centers, a relatively small amount of pulp and almost collapsed blood spaces. In the experimental animal, the lymph nodules are regularly much reduced in size and widely separated by marked to extreme congestion. In three specimens, there are distinct zones of hemorrhage within numerous nodules.

The germinal centers are small, devoid of lymphoblasts and are composed of large, elongate stroma and endothelial cells. These remains of the germinal center, present in about half the thirty specimens, contain small numbers of lymphoblasts in seven instances.

A conspicuous finding in all but four specimens is necrosis in and about the germinal centers, which is manifested by disorganization, swollen disintegrating cells and much nuclear débris. Even in the absence of the germinal center, necrotic lymphoid cells of the nodule are often found. Constantly accompanying the necrosis, there is a response on the part of the endothelial cells, without, however, any polymorphonuclear infiltration. The endothelial cells probably have a local source and assume phagocytic activities, since one can trace such cells, marked by contained brown pigment granules, from their location in the germinal centers of the normal spleen through changes responding to the necrosis of the lymphoid cells, to their own degeneration. Normally they are flat or rounded; in experimental specimens they are often swollen, vacuolated and phagocytic; and in about one-fourth of the specimens, they show necrosis and disintegration.

Satisfactory frozen sections of this organ, stained for fat and counterstained with hematoxylin, were obtained from twenty-eight specimens. After careful search of several sections of the normal spleen, small numbers of fat granules were seen in only two or three endothelial cells of the germinal centers. In the experimental tissues, extracellular granular fat is demonstrated in nine instances in the necrotic areas of the lymph nodules. In the same areas, many endothelial cells containing fine fat granules are found in nineteen specimens and fewer such cells in an additional six tissues.

Not only is there an endothelial response within the lymph nodule, but also about the nodule and in the pulp. In half the tissues of this group of animals, there is a distinct, and in some cases marked, hypertrophy and apparent hyperplasia of the endothelial cells. These cells are large and rounded, their nuclei are commonly weakly stained and occasionally they show degenerative changes. Endothelial leucocytes (free mononuclear cells) are likewise mildly to markedly increased in numbers in about two-thirds of the specimens; they often demonstrate mitoses and rarely are phagocytic. In numerous instances these two classes of cells are especially prominent and numerous in the neighborhood of the lymph nodule.

In the frozen sections no fat is demonstrated in the endothelial cells of the sinusoids of the normal spleen, but in the experimental animals granular fat is stained in many of these cells in about one-third of the specimens.

Polymorphonuclear leucocytes are variable in occurrence. They are conspicuous in the pulp in eleven instances and are in large numbers in six additional cases. As a rule, these cells are scattered and only in one instance are also in foci.

Lymph Nodes: Sections of these structures are available for examination from twenty-two animals. In nineteen cases, they are from the axillary or inguinal regions, and in other instances from miscellaneous sources. While the lymphocytes are not appreciably reduced in numbers, other changes are similar to those in the spleen: the germinal centers are small and often devoid of lymphoblasts; necrosis in the region of the germinal centers is present in over half the specimens and accompanied by enlarged, phagocytic endothelial cells. Several sections of lymph nodules from miscellaneous sources (gastric and duodenal submucous, peribronchial, mesenteric, in the region of the pancreas) likewise show the same degenerative changes.

Phagocytic endothelial leucocytes are common in the sinuses, but are also found in the normal lymph nodes. Congestion is noteworthy in a few specimens, while hemorrhage is not encountered.

Lungs: Recent hemorrhage into small groups of alveolar spaces or in the subpleural region is present in thirteen specimens. This effusion of blood is not massive, no break in the alveolar wall is obvious and an inflammatory reaction does not result. In a few instances when cysts of *Pneumonyssus griffithi* [2] are in the sections, small areas of recent hemorrhage are commonly adjacent. Congestion of alveolar capillaries is not conspicuous, but is found in eight of the thirty specimens and not always coincident with the hemorrhages.

Stomach: Of the twenty-four sections of stomach available for study, only three show small areas of recent hemorrhage in the mucosa and without inflammatory reaction. In an additional three specimens, appreciable numbers of extravasated red blood cells occur in the same location. They are also found on the mucosal surface, usually not coincident with the extravasations in the mucosa, in seven instances. No lesion or erosion is evident in any case, and congestion of mucous or submucous capillaries is neither common nor extreme.

Intestines: Sections of intestine were not examined routinely and only one of the few studied shows an extensive submucosal and less subserosal hemorrhage without inflammatory reaction. No lesion in the mucosa is found.

Adrenal Glands: The outstanding pathology of these structures is necrosis of the cells of the zona fasciculata. This occurs in about half the thirty specimens of the series. The necrosis involving numerous scattered cells to small and large groups of cells, is manifested by loss of staining property, disorganization, and fusion of necrotic cells. Karyorrhexis is common and much nuclear débris is often present. Polymorphonuclears invariably react to the necrosis and are found in large numbers both in the neighboring blood spaces and within the necrotic cells or areas.

Eight specimens show congestion of the small blood vessels. In three cases there are either mild or marked recent hemorrhages in the zona fasciculata and between this layer and the medulla. Almost every specimen, as well as those from the control monkeys, has from two to six peculiar calcareous concretions in a section in the medulla close to the cortex.

Pancreas: This organ is not essentially altered aside from frequent congestion and the acute pancreatitis in one animal (No. 229).

Voluntary Muscle: The variation in staining reaction of the muscle fibers and occasional loss of cross-striations are similar to the findings in the normal rhesus tissue. Likewise, the frequent cysts of Sarcosporidia are present both in the control and experimental animals.

Brain: Six specimens of this organ demonstrate no essential deviation from normal, aside from occasional mild congestion.

Discussion

The type or degree of pathology shown by these animals does not seem to depend on the method by which the virus was transmitted.

Fatty degeneration is a prominent feature in the liver, kidney, heart and spleen. In the first three of these organs, the parenchymatous cells are thus regularly affected, while in the spleen the necrotic areas in the nodules and endothelial cells of the pulp and nodules show a fatty change.

Necrosis is likewise common, involving the liver, kidney, lymph nodules of the spleen and regional nodes, and adrenal glands. Cloudy swelling is more extensive than necrosis, however, in the renal epithelium. Apparently in response to the degenerative changes, polymorphonuclear and endothelial leucocytes are usually increased in the liver, the latter type of cell responds in the lymphoid system,

polymorphonuclears react in the suprarenals and no inflammatory cells are found in the kidney or heart. It seems that these reacting cells are called out by more advanced degeneration, rather than by early degeneration or the presence of the virus, since we find them only in those structures showing marked necrosis, and neither in the kidney and heart nor scattered generally where it might be supposed the virus has been.

Hemorrhage as it occurs in the gastric mucosa, lungs and liver, is recent, without inflammatory reaction and not extensive. In view of the fact that blood effusion is mild and is often found microscopically when not seen in gross, it seems that the extravasation of blood is rather by seepage than by rhexis of a vessel. In two instances (sections of intestine and adrenal gland), however, the hemorrhages are extensive.

No definite evidence is furnished by this study as to the source of the jaundice in these animals. The bile capillaries do not contain inspissated bile and the bile ducts are not remarkably altered. There is a severe disorganization of the parenchymatous hepatic structure, but the endothelial cells of the sinusoids are relatively unaffected. It is noteworthy that although some yellowish brown pigment granules are found in the endothelial cells of the normal splenic pulp, much more pigment is regularly demonstrated in these cells in the experimental monkeys. In frozen sections, the blood spaces are often beautifully outlined by pigment granules.

In the liver parenchyma, an acidophilic, granular type of degeneration is constant and hyaline changes often take place. Nuclei of degenerating liver cells commonly undergo acidophilic changes. While strict midzonal necrosis occurs in only one specimen, the common presence of most extreme necrosis in the intermediate zone and the absence of degeneration of either other zone alone, indicate that the type is fundamentally midzonal and probably begins as such. This view is supported by the fact that the one specimen (No. 304) showing early changes, has undergone fatty degeneration only in the midzonal cells.

The acute degenerative changes of the kidney epithelium and the presence of casts in the tubules indicate the source of the abnormal urine findings of albumin and casts. The occasional presence of lime deposits are likewise of interest.

The sequence of events in the splenic nodule appears to be a dimin-

ution in the number of lymphocytes and lymphoblasts, followed by necrosis of the latter cells and neighboring lymphoid cells, sometimes with fatty changes; the whole is accompanied by a local endothelial activity, and in turn, by fatty degeneration and necrosis of the endothelial cells. Many specimens show small lymph nodules and no trace of germinal centers. While this may be due to the age of the animal, it seems rather that the centers have disappeared in the course of the disease, since at necropsy only a few monkeys were obviously old and microscopic evidence of advanced age is seldom encountered. Lymphocytes are not increased in the blood spaces and the supposition is that the lymphocytes disappearing from the lymph nodules are not diffused through the pulp. The presence of polymorphonuclears is probably in response to the degeneration of endothelial cells.

Special staining for microörganisms in the monkey tissues has been undertaken in three ways, aside from the search in the ordinary paraffin sections. These are Levaditi preparations for spirochetes and leptospiras, Giemsa stains of paraffin sections, and Giemsa, Fontana and other methods of examining smears of fresh tissue. Levaditi preparations, controlled by guinea pig tissues showing large numbers of *L. icteroides*, have not shown any such form in monkeys either running the full course of the disease or killed on the first day of fever (killed monkeys are not included in the series discussed in this paper). Giemsa stains of the paraffin sections have demonstrated bacteria, appearing as postmortem invaders, only in those animals necropsied some time after death. In a limited number of monkeys in which the disease ran a fatal course, in one monkey killed on the first day of fever, and in controls, Giemsa, Fontana and other bacterial preparations were made of fresh smears of the spleen, liver and kidney; no bacterial forms have been found that are not also in the control smears.

Summary

The microscopic pathology in thirty *Macacus rhesus* monkeys fatally inoculated with the Asibi strain of yellow fever virus is described.

In the liver, fatty degeneration, necrosis and nuclear changes are prominent. Polymorphonuclear and endothelial leucocytes are commonly present.

The renal epithelium shows fatty degeneration, cloudy swelling and less extensive necrosis. No inflammatory cells are found. Tubules contain hyaline, granular and, in a few instances, calcareous casts.

Fatty degeneration is an almost constant finding in the muscle fibers of the heart.

The pathology of the spleen includes congestion, diminution of lymphocytes and lymphoblasts, necrosis of lymph nodules and a marked endothelial response in the nodules and pulp. Fat is demonstrated in this organ in the necrotic areas and in the endothelial cells of the nodule and pulp.

Regional lymph nodes likewise show necrosis and endothelial activity.

The lungs and stomach furnish evidence of recent hemorrhage without inflammatory reaction, mild in degree and without obvious lesions in the vessel wall.

Necrosis with a polymorphonuclear reaction is common in sections of the adrenal gland. Mild to marked hemorrhage occurs infrequently.

Tissues so far studied and showing slight or insignificant changes are the brain, pancreas and voluntary muscle.

No bacteria, leptospiras or spirochetes have been demonstrated. The lesions of the various organs may be explained on the basis of a severe intoxication and no nidus of the virus is suggested.

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THE PATHOLOGY OF EXPERIMENTAL YELLOW FEVER IN THE *MACACUS RHESUS* *

III. COMPARISON WITH THE PATHOLOGY OF YELLOW FEVER IN MAN

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The preceding papers [1] have presented the gross and microscopic pathology in the *Macacus rhesus* fatally infected with the Asibi strain [2] of yellow fever virus. The purpose of this paper is to compare these findings with those in human cases of yellow fever.

The literature regarding the pathology of yellow fever in man largely concerns this disease as it occurs in the Western Hemisphere. There are, however, a few references available on this subject from West Africa, including articles by Aitken, Connal, Gray and Smith,[3] Aitken and Smith,[4] Klotz and Simpson, [5, 6] and notes by Stevenson,[7] Turnbull,[8] and Boyce.[9]

GROSS PATHOLOGY

In order to obtain more data on the gross pathology of this disease in West Africa, we reviewed the records of thirty-three cases confirmed by microscopic study, contained in the files of the West African Yellow Fever Commission. These necropsy records are available through the courtesy of the medical authorities of Nigeria and the Gold Coast, British West Africa. The postmortem examinations were made in most cases by the local medical officer or pathologist and occasionally by members of this Commission. The microscopic studies were completed by the pathologists at the Medical Research Institutes of Accra, Gold Coast, and Lagos, Nigeria. In addition, all cases but two were examined microscopically by the pathologists of this Commission with complete agreement as to the diagnosis. We wish to express our thanks and appreciation for the coöperation and interest manifested by the medical administrative officers and by individuals who conducted the postmortem examinations, submitted their records for our use and sent specimens for our examination.

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Except for four Syrians and four native Africans, the patients were Europeans, at the time resident in West Africa. A brief résumé of the reported findings in these thirty-three cases follows:

Jaundice: regularly of the skin and a little less often of the sclerae; commonly of the aorta and other large vessels near the heart, cardiac valves, and subcutaneous tissue and fat; frequently of the costal cartilages, renal tissue, and heart musculature; and less often of other parts and body fluids.

Hemorrhage: as petechiae, in a third to a half of the cases, in the skin, pleurae, epicardium and endocardium, mucosa of small intestine, and beneath the renal capsule; occasionally in the peritoneum, papillary muscles of the heart, bladder mucosa and mucosa of colon; and rarely in the subconjunctival tissue, retroperitoneum and mucosa of trachea. Altered blood ("black vomitus") in the stomach in twenty-nine instances and mucosal hemorrhages in twenty-four; blood in the small intestines in twelve, with mucosal hemorrhages in fourteen; in the colon, blood in four and hemorrhages in three. Hemorrhages and congestion in the lungs in about two-thirds of the cases. Evidence of bleeding gums frequently recorded.

Liver: regularly "boxwood" in color, described as yellow, yellowish brown, yellow-khaki, or reddish yellow; sometimes enlarged; and on section, often friable, fatty, mottled by lobular markings, but seldom congested and hemorrhagic.

Spleen: usually enlarged, congested, firm and malpighian bodies often prominent; malaria in one case (a native African).

Kidneys: enlarged and congested in over half the cases; often icteric; cortex swollen, cloudy and fatty; and blood in renal pelvis in four instances.

Heart: often pale or pale brown; sometimes soft, and certain parts hemorrhagic as described under the subject of hemorrhages.

Bladder: occasionally empty but as a rule containing bile-stained urine, positive for albumin, casts and bile.

Other Organs: adrenal glands, normal; pancreas, occasionally bile-stained and congested; lymph nodes, not remarkable or slightly enlarged; brain (one specimen), mildly congested. Four patients were women; uterine hemorrhage was found in two cases, of whom one was known to be menstruating at the time.

Cutaneous jaundice and petechiae were obviously difficult to determine in the four cases of African natives. Likewise, any abnormal

coloration of the liver and fat in these individuals is confused by the pigmentation caused by the common diet of palm oil. It should be noted that the necropsy observations were made by several investigators and in some cases were incomplete. Taken as a whole, however, the above résumé with articles of Aitken and others,[3, 4] displays the fact that in the gross findings, yellow fever as it occurs in West Africa is not essentially different from yellow fever of the Western Hemisphere. It is not within the province of this paper to go into details as to the comparison of these findings in the two hemispheres, but simply to furnish a basis for comparison with the findings in the *Macacus rhesus* to which, thus far, yellow fever has been transmitted only in West Africa. The reader is referred to articles describing the pathology of yellow fever in the Western Hemisphere, by Boyce,[9] Marchoux, Salimbeni and Simond,[10] Marchoux and Simond,[11] Rocha-Lima,[12] and more recently by Noguchi,[13] Elliott,[14] and Muller and Blaisdell.[15]

Comparison of Gross Pathology of Human and Monkey Cases

Icterus: A constant finding at necropsies of human victims of this disease, was also manifest in the monkeys, but to a less intense degree. Thus while the skin of monkeys was not, as a rule, jaundiced and the tarsal conjunctivae not always deeply colored, a yellow or greenish yellow color of the laryngeal cartilage, large vessels near the heart and body fluids was regularly observed, and other parts were irregularly icteric. It is to be noted that the abnormal coloration was the same in man and rhesus, that is, a lemon yellow and not an orange or brown color.

Hemorrhages: These were found in both human and monkey cases in the pleurae, lungs, gastro-intestinal tract and gums, but were rarely observed in this series of animals in the endocardium and not seen in the skin, peritoneum, retroperitoneal and perinephritic tissues, epicardium, bladder, liver or kidneys. Petechiae were smaller in the animals than were commonly found in man, but in both the hemorrhages were of recent origin. Altered blood in the stomach deserves special mention because of its prominence. In the series of human cases it occurred in almost every instance, while in the monkeys it was found in one-third of the specimens and

was remarkably similar in appearance. As in man, "black vomit" was often found unassociated with mucosal hemorrhages. Likewise, the intestinal contents were colored reddish brown or black by admixed altered blood both in human cases and in monkeys, with perhaps a greater tendency to mucosal hemorrhages of the duodenum in man.

Lungs: Hemorrhagic changes were present in the lungs of the rhesus as well as of man, and the absence of congestion in the experimental lungs, when the necropsy was done immediately after death, probably indicates a difference in posture.

Liver: The color, pallor and fatty appearance of the liver were strikingly similar. The friability and lobular mottling of the liver tissue, when observed in humans, was the same as in the monkeys. Hemorrhagic areas were absent in the animal livers but there was an agreement in the usual dry, bloodless condition of the sectioned tissue. In the human, the liver was reported to be enlarged in some cases; in experimental yellow fever, we felt that the slight enlargement in some instances was within normal limits.

Spleen: The gross findings in the spleen were alike as regards enlargement, congestion and firmness. There was this difference, however: whereas in the human, the malpighian bodies were often prominent and contrasted with the surrounding congested tissue, in the monkey these follicles were usually very small and poorly outlined. It is of interest that in the records of several human necropsies, the lymph follicles were reported as indefinitely outlined and barely visible.

Kidney: Acute degenerative changes in the kidney as evidenced by an enlarged organ and a cloudy, swollen cortex were found in both classes of specimens. Congestion, often referred to in the human cases, was not a conspicuous feature in the monkey kidneys, and hemorrhage was likewise not seen in the experimental tissues. Icterus, common in the monkeys, was often recorded as being present in human kidneys.

Heart: The heart was sometimes pale and yellowish both in man and in the rhesus and the weight was similarly unchanged. As previously mentioned, the cardiac surfaces were often icteric, but the tendency to hemorrhage in these surfaces was greater in human cases.

Urine: The urinary findings were identical, but hemorrhages were not found in the bladder mucosa of monkeys.

The organs that were spared or showed minor changes were the same in human and animal cases: adrenal glands, lymph nodes, pancreas, organs of the neck, voluntary muscle and brain. Data on the condition of human genitalia are incomplete but in the monkey only jaundice of mucous surfaces was apparent.

Summary

It is evident that similar pathologic processes have taken place in the organs of human and rhesus cases of yellow fever. Jaundice, hemorrhage of various parts, "black vomit," pallor, and fatty necrotic changes in the liver, acute degeneration of renal parenchyma, splenic congestion and urinary findings were present in both man and monkey. Although variation existed as to the degree of intensity or extent of involvement of parts, qualitatively the parallelism was striking as regards the icteric color, the recent hemorrhages, and the appearance of the liver, kidney and spleen.

Microscopic Pathology

The histologic pathology of yellow fever has been a subject of considerable study, with particular attention paid to the striking lesions in the liver. Councilman [16] early described the hyaline bodies found in the liver cell. Rocha-Lima [12] has emphasized the midzonal location of hepatic necrosis, while Marchoux and Simond [11] have shown the tendency to fatty degeneration throughout the organs. Seidelin [17] points out that the liver is the seat of constant pathology, in which there is variation in extent of parenchymatous involvement, but in which disorganization of tissues, necrosis and fatty degeneration are conspicuous. As regards microscopic studies of this disease occurring in West Africa, reference should be made to the articles previously cited, by Aitken and his collaborators,[3, 4] Stevenson,[7] Turnbull,[8] and recently by Klotz and Simpson,[5, 6] who have contributed especially to the study of the spleen in this disease.

We have had the opportunity of studying the tissues from thirty cases of yellow fever occurring in West Africa (Nigeria, Gold Coast, Gambia and Senegal). These specimens did not always include all the organs, but at least the organs showing the changes essential for

diagnosis were available. In addition, tissues from ten yellow fever cases occurring in the Western Hemisphere were studied in Toronto, Canada, through the kindness of Dr. Oskar Klotz. From these studies, we can concur in the opinion of Klotz and Simpson,[5] that "no fundamental difference was to be noted in the pathology of fatal yellow fever cases of West Africa and the Americas." It is not the purpose of this paper to deal with a comparison of the pathology of this disease in the two hemispheres, but to compare the pathology of the monkey tissues with that discussed in the literature and studied by us in human cases. The reader is referred to the works of the various investigators enumerated and to an article in preparation by Klotz ("A fuller study of the comparative pathology — will be taken up in another report" [5]) not yet at hand.

Liver: As in the human, the liver (Figs. 1 to 4) of experimental animals shows the most striking lesions. There is the same type of fatty degeneration with the finer particles of fat often in the midzone; in the animal, however, the quantity of fat stained is more than in most human cases. Likewise, a similar type of necrosis exists in the two sets of tissues, both as to being a granular acidophilic form of degeneration of the parenchymatous cells, and as to demonstrating a midzonal location. In the two sets of tissues, the extent of involvement varies from a strict midzone to inclusion of cells almost to the limiting portal area and central vein, but in no instance has there been seen either a periportal or central necrosis alone. The accompanying hyaline type of degeneration is more conspicuous and common in the human than monkey liver. Both man and rhesus livers show jumbled and irregular, necrotic and completely disintegrated cells, with loss of trabecular arrangement but no loss of cell or lobular space. Nuclear changes are similar, with probably more karyorrhexis in experimental specimens and no acidophilic granules in degenerating nuclei in human cases; there is found, however, the same small compact acidophilic nucleus in some acidophilic cells.

The endothelium of the sinusoids is preserved in the rhesus as well as in man and the Kupffer cells are similarly little changed, although more frequently swollen and phagocytic in man. Numerous specimens of monkey liver show numbers of extravasated red blood cells, and only two of the thirty livers studied demonstrate hemorrhages of appreciable degree. It is much more common to find hemorrhages as well as extravascular red blood cells in human specimens. Inflam-

matory cells, of the polymorphonuclear and mononuclear types, are found more regularly in the experimental tissues both intravascular and extravascular. Occasionally, however, they are entirely absent and, conversely, are sometimes found in human cases.

The portal areas of rhesus more than in human livers show variable numbers of lymphocytes and endothelial leucocytes, but the inconstancy of these cells prevents any significance being attached to them. Bile ducts are regularly normal and no inspissated bile is seen in bile capillaries of either set of tissues.

Spleen: The spleen (Figs. 5 to 8) presents interesting comparative pathology in that the following characteristics are similar: congestion, small lymph nodules, and an endothelial response in the nature of enlarged endothelial cells of the pulp, especially about the nodules, and an increase in free endothelial leucocytes. While in man the germinal centers are generally lacking and rarely show necrosis, in the rhesus remnants of the centers are commonly present and necrosis with large phagocytic endothelial cells is regularly seen. Polymorphonuclear leucocytes are found in the pulp in numerous experimental sections, whereas it is uncommon to find them in the other group of tissues. Klotz and Simpson [6] and others have referred to fatty degeneration of the endothelial cells of this organ, and in the experimental animals, fat is demonstrated in these cells lining blood spaces and in degenerated phagocytic endothelial cells in necrotic lymph follicles.

Kidney: The pathology in the kidney (Figs. 9 to 16) is the same in the two classes of specimens, with differences only of degree. Thus cloudy swelling and necrosis of tubular epithelial cells are found, but more of the former and less of the latter change is seen in the monkey kidneys. Numerous acidophilic degenerating cells are common to both groups of sections. Fatty degeneration occurs in the experimental as in the human tissues, both as to involvement of the tubular epithelium and as to the sparing of other structures. However, this type of degeneration is more pronounced in the animal specimens. Both classes of tissues show a consistent absence of inflammatory cells.

Congestion of small blood vessels, particularly of the glomerular tufts, is a regular feature of human cases, but is only occasionally found in monkey tissues. Hemorrhages are not seen in either group. Tubules contain much granular débris and casts of hyaline, granular

and calcareous types, more regularly, however, in human specimens. A type of degeneration of epithelial cells seems to be at least one source of "lime casts" in monkeys, such as Muller and Blaisdell [15] have described in human kidneys. Glomeruli are almost entirely unaltered, but their capsular spaces contain granular débris in specimens from man more than in those from the other group.

Heart: The heart (Figs. 17 and 18) in both sets of tissues shows fatty degeneration of finely granular form although, as in the liver and kidneys, fat is demonstrated as a rule in larger amounts in the rhesus specimens. Likewise, in both tissues, the muscle fibers are sometimes irregularly stained and cross-striations are indistinct, but these characteristics lose their significance when experimental sections are compared with those of control monkeys. Congestion and hemorrhage play a minor part in both human and monkey tissues and inflammatory cells are regularly absent.

Lungs: Sections of lungs (Figs. 19 and 20) show recent hemorrhages without inflammatory reaction, as a rule, however, more frequently and extensively in man. Similarly, congestion and edema are much more commonly found in the human tissues.

Stomach: The stomach has a greater tendency to congestion and hemorrhage in man, but there is the same type of small hemorrhages and extravasation of red blood cells without inflammatory reaction and without obvious lesions in the vessel wall.

Adrenals: Adrenal glands present contrasting pathology in that monkey sections often demonstrate necrosis accompanied by polymorphonuclear infiltration, which was found by us in one human case but is not recorded by others. Frequent congestion and an occasional hemorrhage are common to both classes of specimens.

Pancreas: The pancreas has been equally unaltered both in man and in monkey.

Lymph Nodes: Specimens of lymph nodes are not commonly available for study from human cases and little is recorded in the literature. In the rhesus, the changes are similar to those in the spleen.

Brain: The brain has been infrequently studied and shows little variation from the normal.

Summary

Fatty degeneration of the liver, kidney, heart and spleen is of the same type in man and rhesus, although more extreme in the latter.

Other degenerative changes of the liver, kidney and spleen are likewise similar as to incidence, type and location. Necrosis of the adrenal glands is only rarely seen in the human, although commonly in the rhesus.

In both sets of tissues, inflammatory cells are lacking in response to hemorrhage in any organ and to the degenerative changes in the kidney and heart. Polymorphonuclear and endothelial leucocytes are usually found associated with the liver lesions in monkeys, while seldom in man.

Hemorrhages and congestion tend to be more frequent and extensive in the liver, lungs and gastric mucosa in human cases, but the hemorrhages are alike in being focal, recent and without obvious lesions of the vessels.

Discussion and Conclusions

It should be borne in mind that these papers on the gross and microscopic pathology in the *Macacus rhesus* and comparison with human pathology are based on the use of one strain of yellow fever virus. However, the pathology induced by two other strains we are studying proves to be similar to that reported and discussed in these papers.

The lesions in the *Macacus rhesus*, brought about by experimental infection with yellow fever virus, seem to result from a severe intoxication, as in the case of human yellow fever and recently expressed by Klotz and Simpson.[6] Neither in the monkey nor in man is there any evidence of the localization of the virus. We have been unable to find either in human cases or in experimental tissues any constant bacterial form, or leptospiras or spirochetes demonstrable in Levaditi preparations.

The monkey specimens of the liver tend to confirm the fact that necrosis in the liver is essentially midzonal in type in yellow fever with less altered cells increasing toward the periphery of the involved region. Likewise, as in human cases, when the degenerative changes approach the limiting structures of the lobule, the most extreme necrosis is usually in the midzone.

Klotz and Simpson [6] have recorded that in the spleen there is a sequence of changes involving the lymph follicles, from early enlargement of the follicle due to hyperplasia of the endothelial elements, followed by loss of lymphocytes, to final degeneration of the endothelial cells. We have not observed in human or monkey tissues the first stage given; otherwise, a study of the rhesus sections makes it evident that the process described by these workers is probably correct. A stage of necrosis is obvious in the monkeys, but uncommonly seen in human spleens in which it is possible the stage might have been passed at the time of death. We would add, however, that in monkeys, degeneration and necrosis involves the lymphoid as well as the endothelial elements of the follicles.

The fatty degeneration of the heart muscle fibers and the same and other acute degenerative changes of the kidney in the rhesus monkey add to the evidence for the clinical manifestations of this disease in man.

If experience bears out the hope that the *M. rhesus* is regularly susceptible to the yellow fever virus, this animal will prove to be of incalculable value in the diagnosis of yellow fever in man because of the remarkably accurate reproduction of gross and microscopic lesions.

NOTE: In the photomicrographs, the lesions of yellow fever are compared as they occur in a human case and in rhesus monkeys. The human case is that of H. P., Accra, Gold Coast, diagnosed as yellow fever clinically and pathologically and from whom a strain of yellow fever virus was obtained by inoculation of a *M. rhesus* with the patient's blood. We are deeply indebted to Dr. D. Duff, Deputy Director, Medical and Sanitary Services, Dr. A. C. Paterson, Senior Medical Officer in charge of the European Hospital, and Dr. A. S. Burgess, Acting Director of the Medical Research Institute, Accra, Gold Coast, for records of this case and material for histologic study.

The sections were prepared from formalin-fixed tissues, stained with hematoxylin and eosin and, for the demonstration of fat, with scarlet red and hematoxylin.

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DESCRIPTION OF PLATES

PLATE 94

FIG. 1. H. P. Liver, showing irregular band of midzonal necrosis and mild diffuse hemorrhage. × 55.

FIG. 2. *M. rhesus*, No. 312. Liver, showing fringe of intact cells about central vein (left), beyond which is zone of necrosis infiltrated with inflammatory cells. Periportal cells (right) vacuolated. × 55.

FIG. 3. H. P. Frozen section of liver, demonstrating fat; less fat in necrotic midzone. × 40.

FIG. 4. *M. rhesus*, No. 312. Frozen section of liver, showing less fat in necrotic midzone. × 40.

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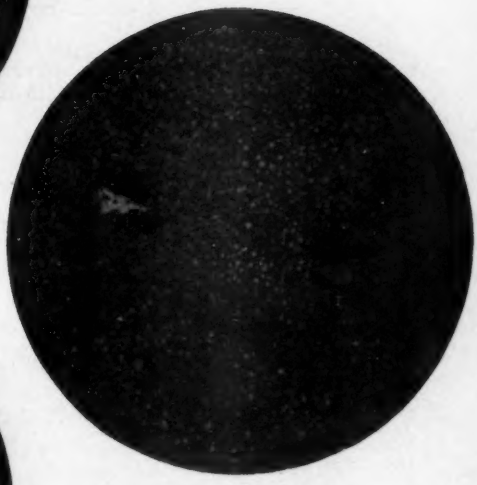

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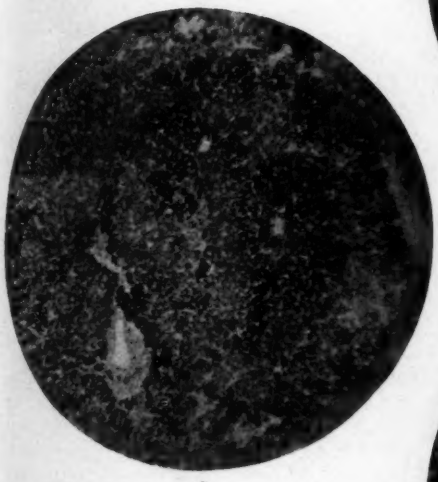

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PLATE 95

FIG. 5. H. P. Spleen, showing small lymph follicle surrounded by faint zone of enlarged endothelial cells, beyond which are congested blood spaces. × 85.

FIG. 6. *M. rhesus*, No. 344. Spleen, showing small lymph follicle surrounded by zone of enlarged endothelial cells, beyond which are congested blood spaces. × 85.

FIG. 7. Spleen of normal *M. rhesus*, No. 315. (Note magnification is lower than previous pictures of spleen.) × 45.

FIG. 8. *M. rhesus*, No. 362. Spleen. Necrosis in lymph follicle. × 150.

5

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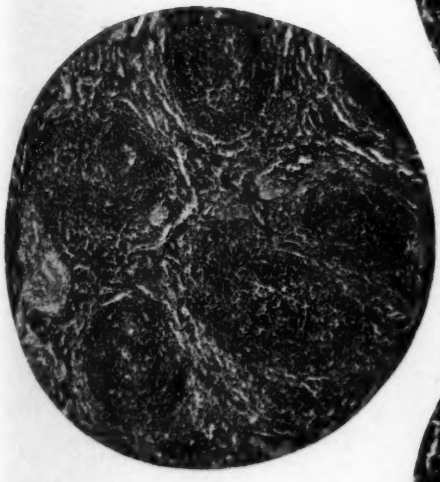

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PLATE 96

FIG. 9. H. P. Kidney, illustrating swollen, acutely degenerated epithelial cells of tubules, and granular débris in lumina. × 150.

FIG. 10. *M. rhesus*, No. 327. Kidney, showing similar acute degenerative changes in vacuolated epithelial cells. × 150.

FIG. 11. H. P. Frozen section of kidney, indicating the presence of fat in tubular epithelial cells. × 150.

FIG. 12. *M. rhesus*, No. 327. Frozen section of kidney, demonstrating much fat in epithelial cells. × 150.

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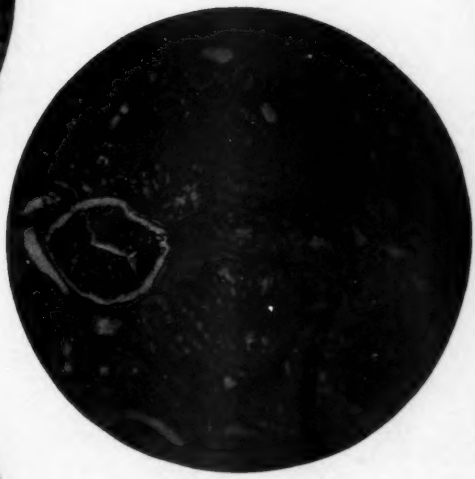

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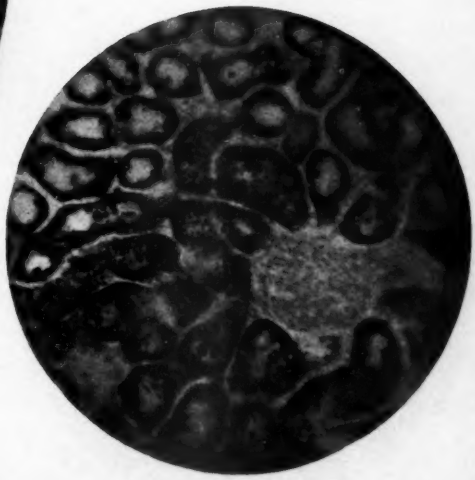

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PLATE 97

FIG. 13. H. P. Kidney section, showing a few hyaline casts (center). Casts not as numerous in this case as is usually found in human kidneys. × 85.

FIG. 14. *M. rhesus*, No. 346. Kidney section, demonstrating several hyaline casts. × 85.

FIG. 15. H. P. Kidney, showing calcareous deposits ("lime casts") on either side of center of picture. × 150.

FIG. 16. *M. rhesus*, No. 253 A. Kidney, illustrating several clumps of calcareous deposits (about center), intensely stained by hematoxylin. × 150.

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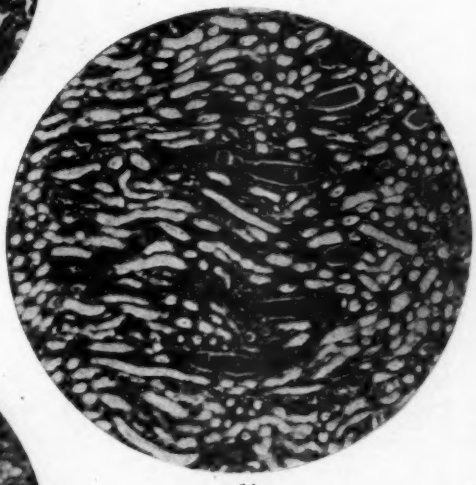

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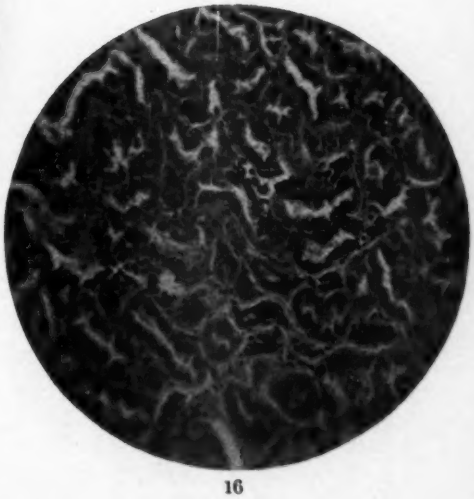

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PLATE 98

FIG. 17. H. P. Frozen section of heart, demonstrating much finely granular fat in muscle fibers. × 150.

FIG. 18. *M. rhesus*, No. 327. Frozen section of heart, showing finely divided fat irregularly distributed among muscle fibers. × 150.

FIG. 19. H. P. Section of lung, showing small area of recent hemorrhage into alveolar spaces. × 85.

FIG. 20. *M. rhesus*, No. 316. Lung section, showing a similar recent hemorrhage into alveolar spaces. × 85.

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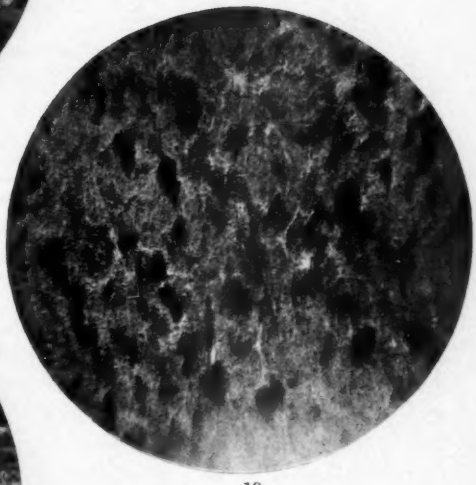

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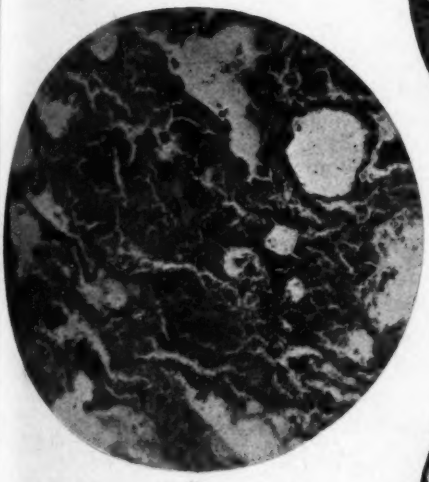

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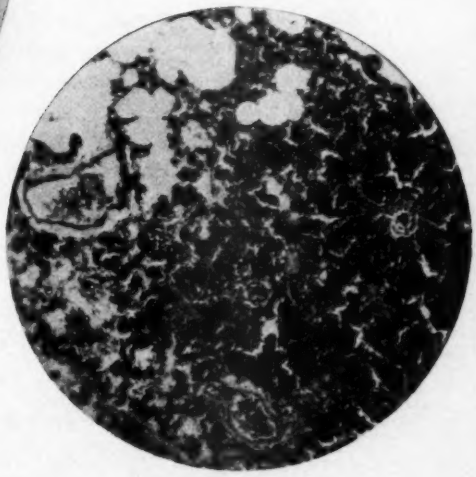

20

MYOCARDIAL DEGENERATIONS IN YELLOW FEVER *

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The nature and mode of production of the toxin in yellow fever is still unexplained. Its widespread effects and influence are seen clinically and can be demonstrated pathologically, in all parts of the body. In the past particular attention has been paid to the degenerations occurring in liver, heart and kidneys. However, as yet, no satisfactory explanation has been given for the part which each of these plays in producing the death of the patient. There is ample evidence, in the clinical course of the disease, that all three of these organs are involved. Jaundice usually appears early in the course of the disease, the sclerae being jaundiced on the second or third day in many cases. The intensity of the jaundice increases with the subsidence of the fever, and may persist well into the convalescent period (Klotz,[1] Elliott,[2] Noguchi,[3] 4th Report of the West African Yellow Fever Commission[4]). As a rule the depth of the jaundice is an indication of the severity of the disease, but in many fatal cases there is little or no jaundice (Klotz).

Uremia is considered by some authors (Elliott[2] and Seidelin[5]) to be the terminal event in all fatal cases. "Albuminuria appears usually on the third day; when it appears on the first day it goes to a fatal termination; on the second day it is a very bad augury."[4] Beeuwkes[6] stated that "albuminuria was by far the most striking feature of this disease; in the Assamenkase epidemic of 1926, in all fatal cases, it was present in large amounts."

Elliott stated that when complete anuria occurs death usually follows in a few hours. In our own series, where histories were necessarily incomplete, one is struck with the frequency of the statement that "complete or almost complete anuria preceded death." Elliott noted the following as manifestations of uremia: air hunger, peculiar whistling respirations, precordial distress, headaches, backache, persistent vomiting, hiccough and finally delirium, Cheyne-Stokes respirations, convulsions and coma. That these are necessarily signs

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of uremia in yellow fever patients, who are already subjects of an intense toxemia and definite heart lesions, is a debatable point. The toxic effect of the disease upon the heart is made evident in three ways, by dilatation, weak soft heart sounds, and disturbances in pulse rate. The damage done to the heart is not purely transitory, nor is it limited to the acute phase of the illness; the heart is so weakened in some cases that death occurs when the patient is apparently convalescent (Seidelin). The disproportion between the height of the fever and the pulse rate (Faget's sign) is observed and reported in most accounts of the disease (Seidelin,[7] Noguchi,[8] Thomas,[9] Elliott, Beeuwkes, and others). This relative bradycardia may be of such extent that a patient with a temperature of 39.5°C may have a pulse rate of 80, and during convalescence a true bradycardia of 32 to 36 beats per minute may develop.

On reviewing the reports of postmortem examinations, one finds that particular attention has been paid to the gross pathological changes found in the heart in yellow fever. The microscopic pathology of this organ has been somewhat neglected, largely because the findings were so disappointingly slight in contrast to the marked macroscopic changes seen in fresh preparations (Otto and Neumann).[10] One of the best accounts of the microscopic cardiac pathology in yellow fever is given by Rocha-Lima.[11] He found that without exception there was more or less fatty degeneration of the myocardium. This was distributed in a somewhat patchy manner but was always more widespread than granular degeneration. On the other hand he seldom encountered myocardial hemorrhages. Nuclei were always well stained and often of strikingly large dimensions; the muscle fibrils and cross striations could always be clearly made out. Vacuolar degeneration, he stated, was well marked but interstitial processes were never noted.

Otto [12] reported varying amounts of fatty degeneration and vacuolar degeneration but no further changes in cells or nuclei. Otto and Neumann could not convince themselves of the fatty degeneration in the subendocardial and subpericardial regions as described by Sodré and Couto. They found that there was nothing remarkable in the muscle fibers. Both cells and nuclei stained well, and as in other organs evidence of inflammatory reactions were lacking.

Seidelin [13] noted a fatty metamorphosis of the myocardium, which was frequently less marked than the gross appearance would lead

one to expect. The aortitis and endocarditis described by Sodré and Couto were regarded as evidence of secondary infection.

Noguchi stated that the muscle fibers showed one or more vacuoles situated in the central portion, suggestive of fat. Certain fibers appeared somewhat swollen. The nuclei were large and vesicular.

Marchoux and Simond [14] noted that in the majority of cases the muscle fibers of the heart were slightly injured by the fatty degeneration, which may be made evident by scant traces of fatty granules throughout the length of certain fibers. On the other hand fatty degeneration may be very marked but was always limited to certain fibers beside which one saw others which were almost perfectly normal. Aitken, Connal, *et al*,[15] reported that in the Lagos epidemic of 1925 the cardiac muscle showed a distinctly cloudy appearance and the fibers contained small vacuoles.

Elliott gave a detailed report of the gross and microscopic pathology observed in Guayaquil, Ecuador, in 1918. He noted that subpericardial and occasionally intramyocardial hemorrhages were found. The muscle fibers were swollen, the striations indistinct, and in the most severe cases entirely absent in places. The nuclei of the most affected fibers were absent.

The pathological findings outlined above were essentially similar in character to the degenerative lesions produced in the liver and kidneys. In the former there were great varieties in the intensity of degeneration, midzonal necrosis being a characteristic feature in typical cases. Fatty deposit was variable and was commonly found in all zones, but in mild cases may be limited to the midzonal areas, and in advanced cases may be most prominent in the portal zone. The distribution and intensity of the degeneration varied in different parts of the liver.

In the kidney, too, there was always degeneration, but the amount of change was not uniform. All stages from marked cloudy swelling to necrosis of epithelial cells were found. Casts and granular débris were seen in the renal tubules. Fatty degeneration was variable, often intensely involving all the tubules of the cortex and medulla. "In general the lesions in these three organs and throughout the body are of a degenerative type, with little or no evidence of proliferative response in fatal cases, and an absence of a primary inflammation" (Klotz [16]).

Outline of Work

The material which forms the basis of this study was placed at my disposal through the kindness of Professor Oskar Klotz. It consisted of sections and blocks of material obtained at autopsy from twenty-nine cases of yellow fever which occurred in West Africa in 1925, 1926 and 1927. In addition sections were examined from ten *Macacus rhesus* monkeys, nine of which died from experimental yellow fever transmitted by direct inoculation from the blood of yellow fever patients or by bites from infected mosquitoes. The tenth monkey was killed and the sections examined as controls for the normal histology of the animal. The experimental and clinical work on these animals was done and reported upon by Drs. Stokes, Bauer and Hudson,[17] and the sections made available for me by Professor Klotz. Paraffin and frozen sections were prepared and stained by hematoxylin and eosin, and with Sudan III respectively. Some sections were specially stained for fat by scharlach R. and Nile blue sulfate stains. In three cases the bundle of His was sectioned, stained and examined in the same manner as the other tissues.

Myocardial Degenerations in Yellow Fever in Human Cases

Two constant features, fragmentation and granular degeneration of the myocardium, were observed in all sections stained with hematoxylin and eosin. The fragmentation of the muscle fibers was greatly increased in most cases in which the autopsy took place some hours after death. It is in part due to postmortem changes, although it may be evidence of the severity of the infection. Granular degeneration was found in all cases, but the amount and extent of it was not constant. It varied from very mild cloudy swelling in patchy areas, to involvement of large areas of the section in a marked granular degeneration. The severity of the degeneration in these latter cases, however, was not always more marked than that in which small patchy areas alone were involved. In mild cases the cross striations were not lost, but in more severe degenerations the fibers were swollen, pale staining and cross striations were lacking. In some sections curious variations in the staining of different fibers were evident. Patchy areas stained a deep pink, with a hyaline-like

appearance, were seen in some areas, whereas the greater number of the fibers stained a faint pink. Nuclear changes were seen in most of the cases examined, and varied in intensity with the amount of granular degeneration. They consisted of changes in the size, shape and staining qualities of nuclei. In cases where granular degeneration was extremely marked, nuclei had entirely disappeared. In some sections some nuclei were twice the size of others in the same area, and while some stained a deep and fairly uniform blue, others took a faint stain and appeared granular, pyknotic or vacuolated.

Small punctate hemorrhages were seen in only a few cases. They varied in size from small collections of five or six cells to clumps containing twenty to thirty red cells. In the cases mentioned the hemorrhages were not scattered diffusely throughout the section, but were limited to focal areas. The blood vessels were markedly engorged in two instances, but this was not a marked feature of the condition.

Inflammatory changes were noted in ten cases, but of these four were old pericardial lesions, and four consisted of thickenings of the walls of blood vessels together with small perivascular collections of mononuclear cells. In two cases, however, the inflammatory reaction was evidently in response to the intensity of the myocardial degeneration. In one of these, Adjei, the cellular exudate was made up of mononuclear cells, lymphocytes, plasma cells and endothelial cells in varying numbers. It was most evident where the granular degeneration was greatest, but was also seen perivascularly throughout the section. In the other case, McMillan, the reaction was limited to one small area, and consisted of focal collections of white blood cells, particularly polymorphonuclear leucocytes, in areas in which granular degeneration was most marked.

So-called vacuolar degeneration was seen in a few instances. This, however, was most evident in cases where the autopsy occurred some time after death. Consequently this vacuolation along with fragmentation is considered to be largely a matter of postmortem change and artefact.

In sections stained for fat it was always present. The extent and distribution of the fatty degeneration was markedly variable. In many cases it was not proportional to, or indicative of, the myocardial damage, as judged by granular degeneration, commonly being more extensive than the latter. In the majority of cases there was a patchy distribution of the fat throughout the fibers, the fatty

deposit being most marked about the nuclei. These fatty changes about the nuclei are not to be confused with the golden-brown granules of brown atrophy which are found particularly at the ends of nuclei. The fat was present in longitudinal rows of fine granules, throughout the length of the fibers, increasing in size to small droplets in the neighborhood of nuclei. In milder cases the fatty degeneration was seen only about the nuclei, and its patchy distribution in the section was markedly emphasized. In severe cases fatty degeneration was very marked, practically all fibers being affected, but even here the patchy distribution of the lesion was noticeable, some areas showing considerably more degeneration than others.

Unsatisfactory results were obtained in sections stained by Nile blue sulfate, since the fat appeared sometimes red, sometimes blue, and all gradations between these two colors were observed. One cannot therefore draw any conclusions from this study as to the chemical nature of the fat.

On examining sections taken from the conducting bundle the results were disappointing. In one case, Mrs. Elmore, there was a marked fatty degeneration of the bundle. In the two other cases no fatty degeneration was observed. One cannot conclude from these findings, however, that fatty degeneration of the conducting bundle was not present in more than one third of the cases. There was only a slight fatty degeneration present in sections of the myocardium from these two cases. We have found that this was not an infrequent occurrence in specimens preserved for long periods in formalin. This was particularly evident in one case where sections were stained immediately postmortem, six months later, and then again eighteen months later. The fat was markedly decreased in the second instance, while in the third set it had almost entirely disappeared. This, however, was not always the case; some specimens seemed peculiarly susceptible to the action of formalin. In so far as could be determined the formalin was the same in all cases.

Summary

Fatty degeneration was observed in all cases. The distribution and intensity of the degeneration varied from field to field in the section, and from point to point in the fibers, being particularly concentrated about the nuclei. The nature of the chemical composition of the fat could not be determined by the use of Nile blue sulfate

stain. The results of sectioning the connecting bundle were inconclusive; further work should be done upon both these points.

Cloudy swelling was a constant feature, but here, too, variations in intensity were marked. Nuclear changes and fragmentation of muscle fibers were features of all cases. Hemorrhages occurred in seven cases, and in these instances were very fine punctate ones. Engorgement of blood vessels was not a marked feature, occurring only in two cases. Previous inflammatory changes were present in eight cases, while two showed inflammatory exudates which had responded to very acute degenerations of myocardial fibers.

Myocardial Degenerations in the Macacus Rhesus in Yellow Fever

The experimental work upon these animals was done by the late Dr. Adrian Stokes, and by Drs. Bauer and Hudson at Lagos, West Africa. A preliminary report of their work is found in the *Journal of the American Medical Association*,[17] and a fuller report in the *American Journal of Tropical Medicine*.[18] The first animal was inoculated directly from a human case of yellow fever, the second and third by transfer inoculations from the first, and the remainder by bites from infected mosquitoes. The clinical course of the disease was similar to that of yellow fever in man, fever occurring upon the third or fourth day following inoculation. Death resulted in from one to seven days after the initial fever. The gross pathology was similar to that of human cases, and the authors were convinced that they had successfully transmitted yellow fever to the *Macacus rhesus*.

The normal microscopic anatomy of the heart of the *Macacus rhesus* differed considerably from that of the human. The myocardial fibers were narrow and closely compacted, cross striations were well marked and easily made out. The nuclei were variable in size and shape but were usually rounded and oval in form, and large in comparison with the size of the fibers. The walls of the blood vessels were thickened. There was also an increased amount of connective tissue with a slight exudate of lymphocytes and endothelial cells about them indicative of previous inflammatory changes. Autopsies were performed immediately after death, the animals either dying of the disease or being killed by a whiff of chloroform while in a moribund state.

Sections were made from paraffin and frozen blocks of tissue. These were stained routinely by hematoxylin and eosin, and Sudan III respectively.

In contrast to human cases fragmentation was not a marked feature. However, cloudy swelling and granular degeneration were present. They varied in amount and distribution, but in severe cases cross striations were obliterated. In some sections there were patchy areas which stained markedly acidophilic, the muscle substances appearing very much like hyaline material. Nuclear changes were present in most cases. The extent of these changes, however, was not so marked as in human cases, variability in the size and staining of the nuclei was observed in normal hearts. Occasional punctate hemorrhages were seen. Inflammatory changes, aside from those seen in the normal heart, were not observed, and vacuolar degeneration was missing.

Fatty degeneration was present in all the cases studied. Its extent was variable and ranged from that seen in one case, in which only a minute quantity was present about the nuclei, to that in others in which the whole section was heavily loaded with fat. The patchy distribution of the degeneration was quite marked. The fat was found more particularly in the region of the nuclei. In instances where it was distributed throughout the fiber it did not have the regular linear arrangement of granules seen in human cases. The fat appeared in the form of fine granules, becoming somewhat more globular about the nuclei.

Summary

Fatty degeneration was a constant feature varying in severity from very slight fatty change to a marked general degeneration. The patchy distribution of the lesion was seen characteristically whether the case was severe or light. Cloudy swelling and granular degeneration were constantly present, but here, too, variations in the intensity of degeneration, and patchy distribution of lesions, were observed. Nuclear changes were present in most cases. Hemorrhages were not a marked feature of the condition, occurring only in two cases. Capillaries containing red blood cells were evident in large numbers in two cases. Inflammatory changes were seen in seven cases, but these were not in relation to the disease and were

not more marked than in the normal animal. Patchy areas of hyaline-like degeneration were seen in three cases. Fragmentation and so-called vacuolar degeneration were absent in these cases.

Comparison of Findings in Macacus Rhesus and Human Cases

The pathological findings in the heart were essentially the same in the human and in the *Macacus rhesus*. Cloudy swelling, granular degeneration, fatty degeneration and nuclear changes were common to both. The intensity of the fatty degeneration was somewhat less marked in the *Macacus rhesus*, whereas its patchy distribution was intensified. The distribution and arrangement of the fat was not so regular here as in human cases, but was similar in that it was most marked about the nuclei. Hyaline-like degeneration was a feature of the changes in the myocardium of the monkeys which seemed to be more marked than in human cases. In no case was the degeneration sufficiently intense to produce an active response of leucocytes such as was seen in two human cases. Fragmentation and vacuolar degeneration were absent in the monkeys. This further strengthens the view that they were largely due to postmortem changes, rather than results of the yellow fever, since these animals were autopsied immediately after death.

Owing to the difficulties encountered in obtaining histories and the late period at which natives seek medical aid, the clinical data in many of these cases are necessarily somewhat incomplete. Eighteen cases occurred in whites, eleven in negroes. Twenty-four cases were males, five females. The average age was 32, the youngest 4 and the oldest 57 years. Two cases occurred in children aged 4 and 5 years respectively. The onset in most cases was sudden and severe, with chills, fever, nausea and vomiting, headaches and pain in the loins. The clinical course of the disease was usually rapid, death occurring in four or five days in the majority of cases, the longest being nine days and the shortest two. Fever, black vomit, melena, anuria, jaundice and slow pulse were outstanding in the clinical signs. Jaundice was usually not very marked before death, but at autopsy after the congestion of the skin had subsided it was made out fairly well. However, in a surprisingly large number of cases, sixteen to be exact, it was noted as being very slight, while in some five cases, only mild

or moderate scleral jaundice was noted. Fever was not excessively high in any case, ranging from 100° F to 102° F in most cases and reaching a maximum of 104° F in one case. Faget's sign, or the disproportion between the pulse rate and temperature, was evident in all cases where these data were taken. This was in keeping with the findings of other observers. The causation of this slow pulse rate has been generally explained by the presence of bile salts in the blood stream. King and Stewart [19] noted that the amount of bile salts in a dose of pig's bile lethal for dogs, if injected alone, will produce neither fall in blood pressure nor slowing in rate. However, they found that the amount of bile pigment contained in a dose of pig's bile lethal for dogs will if injected alone cause death, with slowing of the heart and falling blood pressure. They believed the bradycardia to be a direct result of heightened vagus tone (produced by the action of bile pigments) as atropine restored the rate. They also observed a delay in conduction time between the auricle and ventricle amounting to 2/100 to 5/100 seconds.

More recently, doubt has been cast upon the relation of jaundice and bradycardia. McVicar and Fitts [20] have stated that "bradycardia in jaundice has in our experience proved almost a myth; when it has been observed it has given no clue to the diagnosis." An editorial of the *Journal of the American Medical Association* [21] stated that bradycardia was an infrequent accompaniment of jaundice, except in the intrahepatic type or the so-called acute catarrhal jaundice. Clinically in three cases that have come to our own attention recently, where jaundice and fever had been present, slow pulse was not observed. The first case was that of a young male aged 24 with an unexplained jaundice which was marked in the sclera, palms of the hands and soles of the feet. The temperature was 100° F to 101° F and persisted for five days, the pulse rate varied from 90 to 110 during the fever and subsided with it to the normal rate of 74 to 80. The second case was that of a woman dying of eclampsia with marked jaundice, some fever and a pulse rate of 110 to 120. The last case was that of a young woman aged 28, with subacute yellow atrophy; jaundice was intense (duration seven to ten days), temperature 100° F to 101° F and pulse rate 134. As a consequence of these findings, and the fact that in many fatal cases of yellow fever jaundice was not marked, one must conclude that the slow pulse rate here was not due to jaundice. The finding of marked

fatty degeneration in the bundle of His in one case was suggestive and may be a possible explanation of the slow pulse in yellow fever. Further clinical and pathological studies should be done in order to confirm or disprove this.

The findings in the above series of cases were, in general, in harmony with those previously reported. The presence of inflammatory cells in the heart in response to acute degeneration has not been noted before, although it has been reported in the liver by Klotz. The intensity of the myocardial degenerations occurring in the heart in yellow fever was in itself sufficient to account for death in some cases; but in others it must be taken as only contributary in producing death, which was induced by the general toxemic effect of the disease upon the whole body, particularly heart, liver and kidneys. The pathological findings in the heart were not sufficient to make a diagnosis of yellow fever, but taken together with the changes observed in liver and kidney are satisfactory evidence upon which the diagnosis may be made. The observations recorded in the hearts of *Macacus rhesus* were quite similar to those seen in human cases. These findings taken in conjunction with those in other organs make it apparent that a susceptible animal has been found for yellow fever. Noguchi [22] reported that the *Macacus rhesus* was resistant to leptospira icteroides strains isolated from yellow fever cases in Guayaquil, while marmosets succumbed to the infection with pronounced symptoms. Their findings in these animals were different from those in our report. In hematoxylin and eosin sections the striations were visible but slightly less distinct than normal. The muscle fibers were the seat of numerous very minute vacuoles. Nuclei were normal and no hemorrhages or other forms of degeneration were present. In scharlach R. sections very fine fat droplets were sprinkled uniformly throughout the entire length of all the muscle fibers. There was no accumulation of fat about the nuclei. Similarly Muller [23] reported that there were no hemorrhages or any form of degeneration such as Zenker's. In scharlach R. sections (one monkey alone was examined) numerous fine fat droplets were sprinkeld uniformly throughout the entire length of all muscle fibers. The fact that these findings were at variance not only with those in human cases, but also with those found experimentally in our series of *Macacus rhesus* suggests that the infection under these different conditions was not the same.

Summary and Conclusions

1. The microscopic examination and analysis of the hearts of twenty-nine cases of West African yellow fever, and those of nine monkeys experimentally infected with West African yellow fever, is here reported.

2. Cloudy swelling, granular and fatty degeneration were found constantly in the hearts of both the human cases and those experimentally induced in the *Macacus rhesus*.

3. Primary inflammatory changes were not seen in the heart in yellow fever. Secondary response of white blood cells to intense degeneration was observed in two human cases.

4. The distribution and intensity of granular and fatty degeneration was patchy and variable in both human cases and *Macacus rhesus*. Fatty degeneration was most marked in the neighborhood of nuclei of the fibers.

5. The causation of the slow pulse in yellow fever is still uncertain, and doubt is thrown upon the belief that it is due to the jaundice.

6. Further investigation of the clinical function and the pathological changes in the bundle of His may lead to solution of the problem.

7. The lesions in human hearts and in those of the *Macacus rhesus* are essentially the same.

8. The lesions in the heart are in themselves not sufficient to make a diagnosis of yellow fever.

In conclusion I wish to thank Professor Oskar Klotz, at whose suggestion this work was undertaken, and who has greatly assisted me during its prosecution by advice and direction.

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CORPORA LIBERA IN THE TUNICA VAGINALIS TESTIS *

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In dissecting room cadavers one not infrequently observes calcareous areas in the visceral tunica vaginalis and the albuginea. Since most of the people whose bodies are dissected here were in the later decades of life — fifty to eighty years — the processes responsible for the condition long have ceased to be active, and one is left to rely on inference. Although I do not recall ever having seen adhesions between the parietal and the visceral portions of the tunica vaginalis in these calcified regions, as mentioned by Reclus,[1] the areas apparently have a traumatic and inflammatory origin. One frequently meets with adhesions, but for some reason I have not observed calcification in them, although calcification in cases of *concretio pericardii* is not so rare. Nor have I seen ossified plaques such as that described by Cohn.[2]

Most of the calcareous areas which I have seen occurred in the visceral portion of the tunica vaginalis only. They were small, though some measured 6 by 10 mm. The plaques usually were scale-like, rather irregular in form, and the calcification never extended far into the depth, seemingly being confined mainly to the two tunics — the visceral vaginalis and the albuginea. The testicles upon which the calcareous areas were found seemed grossly normal otherwise. If inflammatory processes are factors in the genesis of these plaques, one might assume that both the parietal and visceral portions of the tunica vaginalis would be similarly affected, but it is possible that the difference can be attributed to the fact that the fibrous layer of the parietal portion is relatively loose, while the extremely thin visceral portion overlies the densely fibrous and relatively thick albuginea. Another reason may lie in the fact that the inflammatory processes concerned probably take their origin from within the testis or epididymis. Park[3] gave a good summary regarding this condition and quoted from a number of older writers. Keyes,[4] who gave a skiagram of a case of very extensive calcification in the tunica vaginalis, says that he "twice met with calcification of the vaginalis, a very rare condition, which was exhaustively described by Roswell

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Park." It seems strange that Keyes saw calcification only twice in an apparently large clinical experience, for it is not at all uncommon in dissecting room cadavers.

Probably more common than the occurrence of calcified areas on the testis, is the presence of free bodies in the cavity of the tunica vaginalis. I have not kept accurate count of all the cases I have seen, but recall approximately a dozen with free bodies. It is highly probable, however, that many cases were overlooked until I began to direct my attention particularly to the condition. These free bodies varied from tiny granules to about 4 mm. in diameter, and they usually were somewhat flattened, though sometimes quite spherical. A few were found lying in slight depressions on the epididymis, and one was similarly placed below the inferior pole of the testis. Some lay in the sinus epididymis, which, when well formed, is a very convenient receptacle. Most of them lay free in the cavity of the tunica vaginalis and made no particular imprint on the testis because they were small or flattened. I have never found more than four in one cavity, but have just recently found the seven shown in Fig. 1 in four out of twelve bodies, the tunicae vaginales of all of which were carefully opened and scrutinized.

Some of the larger bodies did not feel very firm to the touch, but others, even of the smaller, were so hard that they rebounded as they were dropped into a small glass vial. The testis represented in Fig. 2 contains three of the larger of these free bodies and an additional one probably lay in the depression evident on the caput epididymis. Unless students are cautioned in advance, and the greatest care is used in opening the tunica vaginalis, not only the small but also the large free bodies drop out and escape attention, and from recent observations I feel quite convinced that they are present far more commonly than I had surmised. They may be quite well known, but a fairly comprehensive search of handbooks and text books on pathology and surgery, both general and genito-urinary, both past and recent, has provided me with but a few references.

In his comprehensive treatise on pathological anatomy, which appeared in four fine royal folio volumes, two of which are composed of exquisite plates, many of which are in color, Lebert [5] says that Luschka thought that cartilaginous bodies of the tunica vaginalis arose from the fringes and appendices of the tunica vaginalis. Lebert held that these bodies are neither cartilaginous nor osseous,

but fibromata with calcareous centers, and he quoted Duplay (reference not cited) as saying that he had seen small cartilaginous or osseous tumors which were attached to the albuginea in the region below the caput epididymis and floating free in hydrocele fluid.

According to Duplay, these bodies vary in size from a hemp seed to a cherry, and in the quotation given by Lebert the former states that he saw small free, cartilaginous bodies with a calcareous center in four cases of hydrocele. In one of these a similar small body was still attached by a filiform pedicle in the region below the head of the epididymis. Duplay wrote that these free bodies arose from cysts which he thought might form anywhere on the testicle and later become detached. He believed that irritation of the serosa by such detached bodies would cause hypersecretion and account for the commonness of hydrocele in later years.

In discussing chronic vaginitis testis, Koenig [6] stated that calcification occasionally occurs upon the testis in this condition, and then added that it is particularly in these cases one frequently finds pedunculated or free, firm bodies in the tunica vaginalis.

I am indebted to Dr. L. A. Sigurdson for calling my attention to a statement by Keyes upon this matter. Under the head of "Fibrous Bodies," Keyes wrote: "The so-called fibrous bodies occasionally met with upon opening a hydrocele are concretions of earthy phosphates or carbonates covered with fibrin. Probably they are for the most part due to a deposition of the hydrocele salts upon some warty growth, followed by atrophy of the little nucleus, after which the concretion breaks free. Wendlung met with concretions six times in 109 operations (Péraire). They do not exceed the size of a pea — though Chassaignac found one 2 cm. long and 12 mm. wide — and are usually single."

The mode of formation which Keyes suggests for these fibrous bodies in hydrocele, if correct, implies that they belong to a different group than those here considered. I never have seen "warty growths" on the testis and do not know what is meant thereby.

Dr. Sigurdson further called my attention to the fact that Duckworth [7] mentioned the presence of a calculus in a eunuchoid cadaver. Unfortunately one cannot be certain regarding Duckworth's description, for when speaking of the testes of this cadaver he merely says "in each case a hydatid body is present, and at the base of one testis

an extremely hard calcified nodule of the size of a grape stone was discovered." Duckworth unfortunately did not state that this calculus was free in the cavity of the tunica vaginalis, but if it was it may belong to the group here considered.

Péraire [8] stated that free bodies are rare and had scarcely been described, but added "now that resection of the tunica vaginalis is performed for the radical cure of hydrocele, they will no doubt be found more frequently." Péraire apparently overlooked the fact that his fellow-countryman, Reclus, had emphasized that they are not rare, as Damaschino [9] had stated. Reclus found them in fourteen out of 260 cavities of the tunica vaginalis. He stated that they frequently are the size of a grain of millet or of hemp, and added that their structure has long been known and also their mode of formation. According to Reclus, they are fibrous and one can observe their mode of formation on the same testicle. He stated that at first a little prominence appears which looks like a whitish spot, (*point laiteux*). Later a pedicle is said to form and when this breaks the body becomes free. He stated that three or four bodies as large as a grain of sand may be found on each hydatid; and also that pendulous free bodies are sometimes found embedded which can be expressed by force through a narrow opening or by breaking the overlying membrane. He thought that they were never found except in cases of hydrocele, and said that Mallassez found free bodies in which blood resulting from hemorrhage was still present.

It is not improbable that Reclus's statement is based on the description and mode of origin of these free bodies given by Virchow,[10] according to whom proliferation may occur on the testis or epididymis without the presence of hydrocele. Virchow spoke of warty outgrowths or excrescences which were flat and lobulated or pedunculated and had a tendency to become thick and spherical at the ends. According to him, calcification occurs early and they may become larger through the addition of concentric layers, and may have the form of papillae and villi, which may be branched. He stated that they become cartilaginous in consistency and are more common in moderate grades of hydrocele and rarest in severe cases. He found them easily palpable and stated that on section he found an outer partly cartilaginous layer and an inner calcific nucleus. In free bodies as large as a cherry stone the calcific center was said to be as large as the cherry kernel. Virchow further stated that the places on

the testicle where the pedicles were attached were indicated by prominences or depressions. He found the bodies to vary in size from the head of a pin to the "bullet of a gun," or cherry stone apparently.

Soon after the publication of Virchow's lectures, Damaschino reported three free bodies found by Legroux through "a happy chance," as he says. The largest of these was 12 mm. in size and almond-shaped. All three were said to have been ivory in color, smooth, elastic and looked like fibrocartilage with a calcified nucleus. One of them apparently was bosselated.

Damaschino recognized two methods of formation, but stated that he was unable to account for the formation of some of them. According to Damaschino, Hunter and Velpeau believed that they arose from blood clots which underwent a fibrocartilaginous transformation, and which, once begun, continued even after the bodies were detached. Damaschino found some of them to contain true cartilage cells and smooth muscle, but this statement probably rests on impressions obtained from naked eye, not from microscopic examination, and belongs in the same category as the suggestion that bodies which exist detached in the tunica vaginalis may continue to grow through their *vie propre*, as Damaschino believed.

In a chapter entitled "On the Formation of Cartilaginous Bodies in the Tunica Vaginalis," Cooper [11] stated that they occasionally occur in cases of hydrocele. According to Cooper, they may still hang from some portion of the membrane and appear to be wholly cartilaginous, although they are that only on the outside, the center being "earthy." Cooper saw them first in the course of dissection in a case of hydrocele, and one of the illustrations accompanying his chapter represents a testis with calcification in the visceral tunica vaginalis, and very small bodies with relatively long, thin pedicles attached to the head of the epididymis. Another illustration represents a testis with the usual appendix testis and epididymis; and a third illustration represents a testis with a thick, calcified plaque in the visceral tunica vaginalis. Cooper apparently was misled regarding the structure of these free bodies by their outward appearance and consistency, and believed that they arose (1) from pedunculated bodies attached to the walls, and that they were covered by reflected portions of the membrane; and (2) from cysts which were said to occur between the visceral tunica vaginalis and the albu-

ginea. According to Cooper, these free bodies cause chronic inflammatory changes in the tunica vaginalis.

The statement of Keyes seems to be based largely on Péraire. The calculi reported on by Péraire occurred in cases of hydrocele of a half to two and a half years standing and were discovered accidentally by palpation after incision of the tunica vaginalis. They varied from a lentil to a pea in size and were said to have a watery organic outer layer which did not dissolve in hydrochloric acid. Yet it was said not to be "organized" and was found to contain "azote" and "globulo-fibrine." Double calcium magnesium phosphate was present, but iron and calcium oxalate and cholesterin were absent. The murexid test was negative.

Most of the free bodies which I have examined so far were practically or wholly calcified, as illustrated by the skiagrams in Fig. 3. Fig. 4, which is a skiagram of the entire testis shown in Fig. 2, indicates that calcification was relatively slight in the three bodies associated with it, although they were among the largest encountered so far. It is interesting that some of the smallest free bodies were found to be the hardest, although a good deal of fibrous tissue was still contained in all of them. Some were sufficiently mineralized to throw full-sized shadows, and it would seem that these should show in skiagrams of the testes taken in the living. The larger encountered by me should also be palpable in the living and might easily give rise to mistaken interpretations in skiagrams of the testis.

A microscopic examination of ten free bodies showed that some are fibrous in nature and others locular. (See Figs. 8, 9 and 10.) As shown in Figs. 5 to 9, inclusive, the interior usually is thoroughly calcified, even when the outer portion is composed of dense hyaline, degenerated connective tissue. In some cases the outer layer was partly calcified and threw as dense a shadow as the rest. Wherever the calcification was the completest, the tissue was most degenerate. None of the bodies contained a cavity lined by epithelium. As the appearance of the section of a calculus shown in Fig. 7 suggests, this might have been sessile and have become detached from the body of the testis after it was calcified, although the fibrous outer layer might also have been torn in the handling.

Although most of them have such a structure as just indicated and represented, other forms such as represented in Figs. 8, 9 and 10 also are encountered. In these the calculi are contained in locules,

each of which is formed by a framework of connective tissue. Such a structure as that represented in Fig. 10 suggests that the free body had its origin in one containing a group of tubules as contained in the appendix testis shown in Fig. 11. The epithelium of the tubules and the contents apparently degenerated and became calcified, the connective tissue septa, although also degenerate, being preserved longer. Such calculi as those shown in section in Figs. 8, 9 and 10 suggest that the larger calcified areas found in other free bodies may have resulted from the fusion of similar smaller calculi and a repetition of this process could result in the formation of a single calcified area contained in a connective tissue capsule. However, if this were the only method of formation, it would be difficult, if not impossible, to account for free bodies with such thick connective tissue capsules as that represented in Fig. 5.

In considering the origin of these bodies, the appendices of the testis and epididymis naturally first came to mind. They seemed the most likely source, although calcification of coagulum also was considered until the microscopic examination seemed to make such an origin unlikely. Since one rarely finds small cysts containing clear, watery fluid in the epididymis and the testis, it is evident that calcification of their contents might also be a source of free calculi. With degeneration of the overlying tunics they could become discharged into the cavity of the tunica vaginalis, but it would seem that a depression should then be found upon the testis in the place where they had formed, but this never was the case. Although small phleboliths are relatively common in the pampiniform veins opposite the testis, it is difficult to see how they could gain entrance to the cavity of the tunica vaginalis.

Since calcification is so common in the human yolk sac of full-term placentae, I expected to find the attached appendices of the epididymis and testis calcified with equal frequency, but I do not remember having found a single case in which the calcification was plainly evident by palpation of the appendices. Microscopic examination nevertheless showed that calcification is common. Many of the appendices, especially those on the testis, are extremely small, and since they sometimes are multiple, the presence of calcification in them made such an origin of the free bodies much more likely. One obstacle, however, is the difference in shape between the free bodies and these appendices. The testicular appendages usually are

ovoid, flattened and extremely thin, and those of the epididymis usually much larger, very soft and not infrequently pyramidal. Moreover, appendices with a thin, long pedicle are relatively rare, and it is difficult to see how sessile appendages can become detached. Nevertheless, a histologic study of testicular and epididymal appendices gives support to the inference that they are the source of the free bodies described above.

Six of the appendices testis and six of the appendices epididymis examined, were vesicular and contained calcareous contents; and fifteen and sixteen respectively were fibrous throughout, or almost so. Most of the vesicular or true hydatid type of appendices were composed of a thin, fibrous wall lined by columnar epithelium surrounding a relatively large cavity containing calcareous material, as shown in Fig. 12. The fibrous appendages, on the other hand, were usually composed of loose, relatively vascular, fibrous connective tissue, which often was surrounded by a fairly well preserved, high cubical mesothelium. Some of these fibrous appendages were decidedly plicated, as shown in Fig. 13, giving the impression in cross-section that they contained a number of cavities lined by cubical epithelium. Others were composed partly or almost wholly of a series of tubules, as shown in Fig. 11, and it is probable that the examination of a still larger series would reveal others with such a structure.

Unless the vesicular appendages, such as that shown in Fig. 12, obtained a very much thicker fibrous capsule before calcification begins, it is difficult to see how they could give rise to free bodies with a structure such as represented in Fig. 5; and, although such fibrous appendages as represented in Fig. 14 may become calcified from within, it is not so easy to see how they could form free bodies with a structure such as that shown in Fig. 5. It is significant, however, that I found one testicular appendage only about 1 mm. in diameter which had practically this structure, and this made such an origin of these free bodies very probable. Since some of the appendages examined were composed of fat, it is possible that some of the free bodies arise from degenerate, calcified, small lipomata, and others from small pedunculated fibromata, although I do not exclude such an origin as postulated by Virchow.

Although it has been suggested in connection with calcified free bodies in the peritoneal cavity that they may become encapsulated

after they have become detached, no one, so far as I know, has brought forward adequate proof to this effect. I am not familiar with the exact cellular contents of the serous fluid in the tunica vaginalis, but it is highly probable that it is similar to that of the peritoneal cavity. Since the conditions for tissue culture would seem to be ideal, it is possible, although not probable, that detached mesothelial cells may revert to a fibroblast stage and, becoming attached to a small calculus or clot, lead to its encapsulation. Moreover, if connective tissue fibers can arise from fibrin, as Baitsell [12] claimed on experimental grounds, the idea of Damaschino that they grow *par leur vie propre* after they are detached, seems less strange, although a source for the cells would still have to be found.

Although I have not examined a large series of free bodies from the peritoneal cavity, I have seen some bodies, both calcified and uncalcified, but they look quite dissimilar to the free bodies found so far in the tunica vaginalis. This statement is based on only three free bodies in the omental bursa and a few small calculi from the rest of the peritoneal cavity, however. Some of the former measured over 1 cm. in length and had a thick, fibrous wall surrounding yellowish, friable contents which apparently were partly calcified. I briefly considered their origin in 1915,[13] and I never have found any evidence suggesting the transformation of free coagulum into connective tissue or the continued growth of free bodies in the serous cavities. Hence, without precluding another origin, I have no reason to assume it until I find free bodies in the tunica vaginalis which have a structure unlike that of appendices testis and epididymis.

It is probable that pedunculated appendices of the testis or epididymis, which do not possess an adequate blood supply, can undergo degeneration and become detached. Since the pedicles sometimes are very fine and relatively long, it is also possible that torsion may be a factor. Some pedicles contain relatively large blood vessels, but others are quite avascular. Since the latter would be dependent upon absorption for nutritive material, degenerative changes would probably appear early.

The fact that one or more free bodies may be present, even in cases in which the appendix testis and epididymis still are attached, further suggests that these appendices often must be multiple. An examination of a sufficiently large number of fetuses and newborn should confirm this assumption.

It does not seem probable that inflammatory changes play a rôle in the detachment of appendices or that such changes can play a part in the formation of the free bodies here described, unless they excite proliferative changes, as stated by Virchow. Since many of the free bodies are so very small, such an origin as this would seem to be precluded in their case, and those as large as a centimeter, reported by others, probably have both a different origin and structure than those here reported.

NOTE: The poor preservation indicated in the cross-sections is due to the fact that all this material was removed from dissecting room cadavers approximately a year after death and preservation. The mesothelium apparently is desquamated before the appendices become thoroughly calcified, and hence is not evident in any of the cross-sections of the free bodies represented in Figs. 5 to 10, inclusive.

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DESCRIPTION OF PLATES

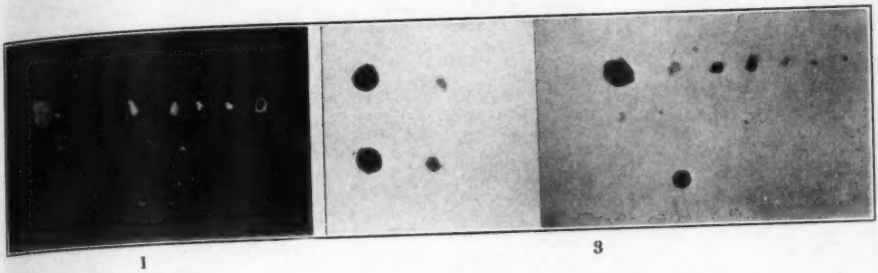
PLATE 99

FIG. 1. Photograph of seven free, calcified bodies found in the cavity of the tunica vaginalis testis. The second and last were so dark in color that they show but faintly in the white circles. Natural size.

FIG. 2. Photograph of testis with three free bodies. One probably was lost.

FIG. 3. Skiagram of eleven free bodies, all of which gave almost full-sized shadows.

FIG. 4. Skiagram of the testis shown in Fig. 2, showing that the free bodies contained only small calcified areas.



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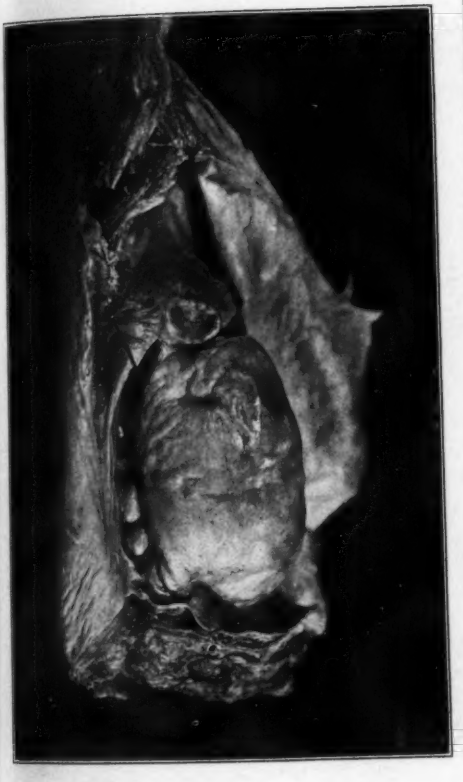


2

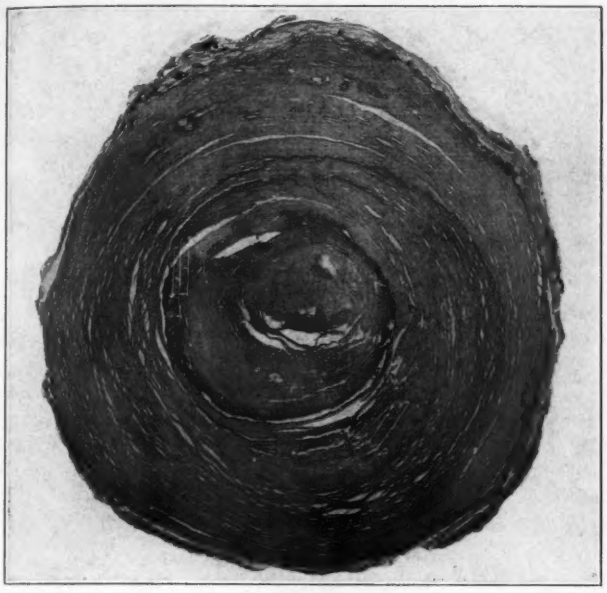
4

Meyer

Corpora Libera in Tunica Vaginalis Testis

PLATE 100

FIGS. 5 to 7, inc. Calcified, free, fibrous bodies in microscopic section. Figs. 5 and 6 × 40. Fig. 7 × 35.



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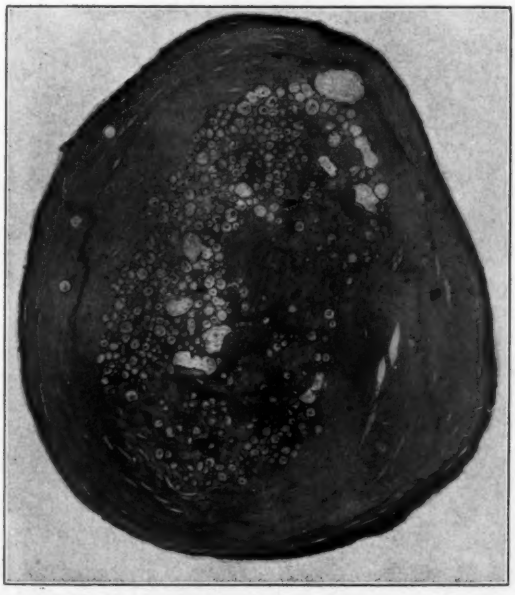

6

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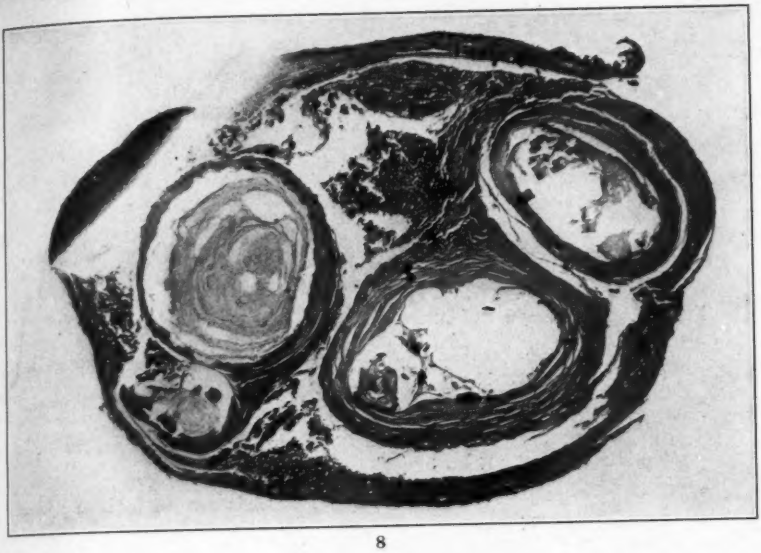
Meyer

Corpora Libera in Tunica Vaginalis Testis

Plate 101

Figs. 8 to 10, inc. Multilocular, partly calcified, free bodies, each containing numerous calculi, the larger of which seem to be fusion products. Fig. 8 × 50. Fig. 9 × 70. Fig. 10 × 40.



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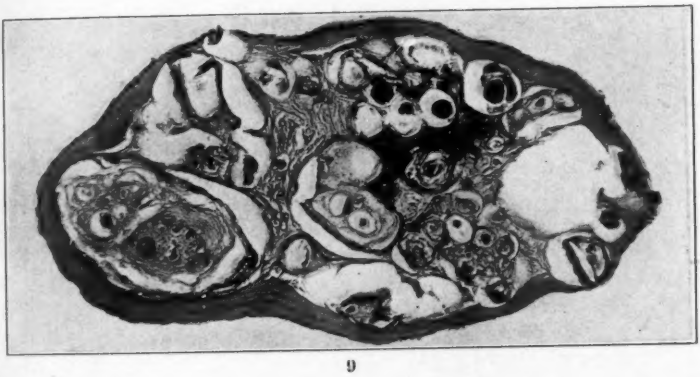

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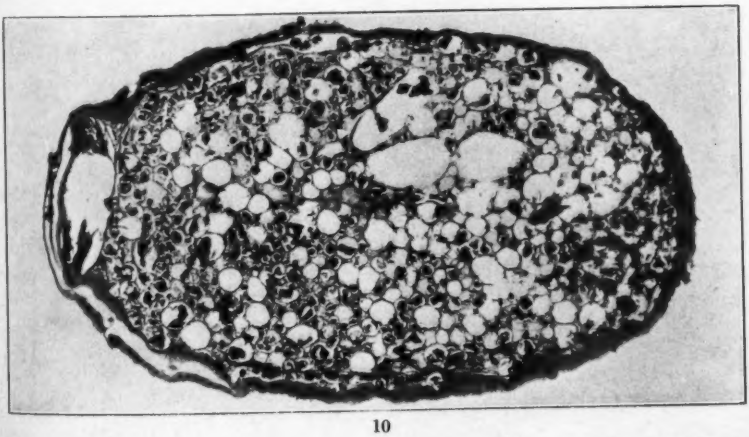

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Meyer

Corpora Libera in Tunica Vaginalis Testis

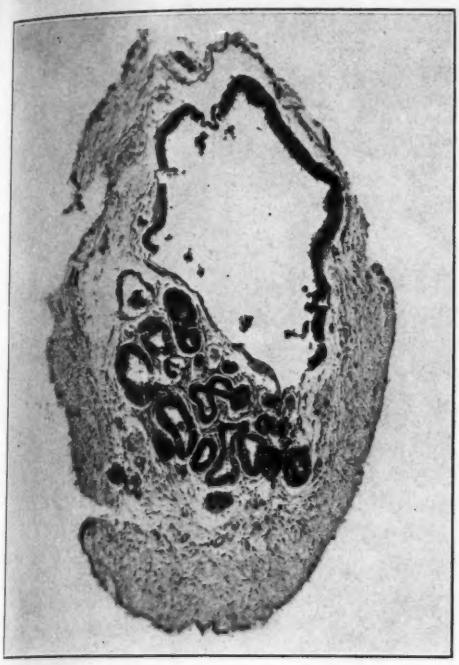
PLATE 102

FIG. 11. A cross-section of an appendix testis with numerous tubules. × 60.

FIG. 12. A cross-section of an appendix testis as usually described, representing the hydatid type. × 50.

FIG. 13. A cross-section of a plicated, fibrous form of appendix still covered with mesothelium. The latter is high cubicle and even columnar in type in the invaginations. × 35.

FIG. 14. A cross-section of a fibrous appendix testis covered by high cubicle mesothelium. × 90.



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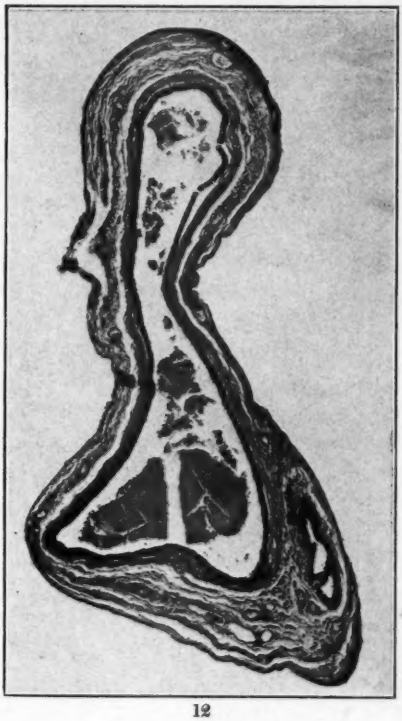

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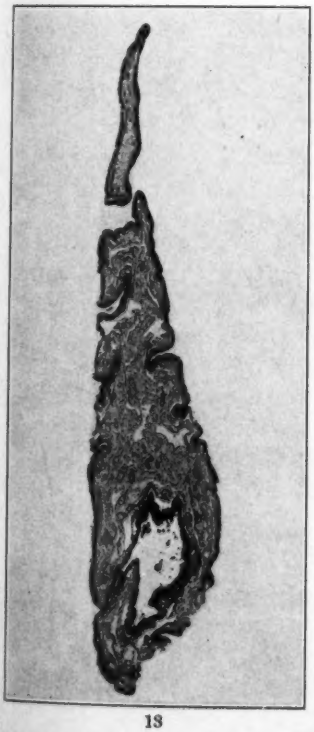

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14

Meyer

Corpora Libera in Tunica Vaginalis Testis

CALCIFICATION OF THE SUPRARENAL GLAND *

BERNARD SELIGMAN

(From the Division of Laboratories, Montefiore Hospital, New York, N.Y.)

Calcification and ossification are extremely rare in the human suprarenal gland. Brüschweiler [1] noted calcification in the suprarenals of fifteen out of 303 cats. Marine [2] reported sixty-four instances in 257 felines and suggested that the toxin of distemper caused focal necrosis of the cortex with subsequent calcification. The process in both instances was bilateral and no ossification occurred. Kruse [3] noted bone formation in the medulla of the suprarenal gland of a monkey. In humans, calcification has been noted in tuberculosis of this gland (MacCallum,[4] Kovács [5]). Wooley [6] described a case of bone and bone marrow formation in a case of tuberculosis of the suprarenal. Tubercles were present around and in the areas of bone formation. Hemorrhage with subsequent calcification has also been noted (Adami and Nicolls,[7] Surbek,[8] Victor [9]). In a boy 14 years of age, who died of malignant lymphogranulomatosis, Harbitz [10] reported calcification of the liver, lungs, kidneys, suprarenals, lymph nodes and capsule of the thymus. Newsam [11] noted ossification of the entire medulla of both suprarenals with marked thinning of the cortex in a girl of 2 years and 7 months who suddenly developed signs of suprarenal insufficiency with fatal termination. Harbitz mentions a case of a deposit of calcareous material with degeneration of suprarenal parenchyma in a case of parathyroid hyperplasia.

Marine found no evidence of calcification in the reticular layer of the cortex in the pig, ox, sheep, dog, rat or rabbit corresponding to the type he found in cats. He thought that the toxin of distemper was the specific cause. Freezing with ethyl chloride and injury to the gland with diphtheria toxin or arsenic gave negative results in the rabbit and cat. Experimentally, calcification has been produced in the kidney tubules, stomach, lungs, and myocardium by intravenous injection of calcium (Tanaka [12] and Katase [13]), overdoses of parathyroid hormone (Hueper [14]) and by acid diets (Dreyfuss [15]). The suprarenal gland has never been the seat of calcification in any

* Received for publication June 4, 1928.

of these experiments. In "metastatic calcification" also, the suprarenal has not been involved in any of the reported cases.

In this report we have collected the human cases having a distribution of calcium deposits in the suprarenals similar to that found in cats by Marine and Brüschweiler. All the material was obtained from the autopsies at Montefiore Hospital. The patients were practically all adults who died from chronic diseases. In none of the forty-six cases of tuberculosis of the suprarenals in our series was calcification found. It occurred, however, in a number of instances in the blood vessels and also in areas of infarction in several suprarenal glands. The routine examination of the suprarenal glands in 1185 autopsies revealed four instances of calcification of the reticular layer similar to the lesions described in cats by Marine. These are here reported. The process was diffuse in three instances and in the other only a spicule of calcium was found. Very little connective tissue or other signs of chronic inflammation were found. Sections of the other gland (in two instances) revealed no other deposits of calcium salts. A soft deeply staining calcium deposit was interpreted as of recent origin and hard pale staining, lighter blue areas were assumed to be older deposits.

CASE 1. *Clinical History:* M. K. Autopsy No. 3419, male, 60, Russian. No history of infectious disease, and apparently was well until three years before his death when all his teeth were extracted on account of ulcerating tissue of the mouth. This ulceration proved to be due to a tumor. He received X-ray and radium therapy. Course of his disease was afebrile.

Anatomical Findings: Carcinoma of the soft palate with metastases to the bones of the skull, calcified tuberculosis of the bronchial lymph nodes, bronchopneumonia. The kidneys showed large calcareous deposits in the collecting tubules. Calcification was present in the intima of the aorta.

Suprarenal: Large area of calcium deposition in the reticular and fascicular layers of the cortex arranged in dense clusters of fine dots and strands around the cells. The calcium in some areas was deep staining. No inflammatory exudate or increase in connective tissue noted.

CASE 2. *Clinical History:* J. L. Autopsy No. 3837, male, 49, born in United States. Mother died at 29 of a "heavy cold." One brother died at 6 months. One sister died at 1½ years. Married at 41. Several children alive and well. Pertussis, malaria at 16, syphilis at 26. He constantly held his pipe in the lower left angle of his jaw. All his teeth were extracted during the course of his illness. Patient ran a slight fever (never over 101°F) for several months. Wassermann ++++, blood sugar 127 mg., urea 11.2 mg., uric acid 3.7 mg.

Anatomical Findings: Epithelioma of cheek, with involvement of the maxilla, luetic aortitis, bronchopneumonia.

Suprarenal: Microscopic area of calcium deposit in the reticular layer of the cortex, arranged in form of dense strands, light stain. No evidence of inflammatory reaction.

CASE 3. *Clinical History:* F. F. Autopsy No. 4195, male, 44, Russian. Previous history not obtained in detail. For one and a half years he complained of diarrhoea, weakness and loss of weight. Physical examination revealed a cachectic individual who had a colostomy. Course was afebrile.

Anatomical Findings: Carcinoma of the rectum with metastases to the liver, spleen, regional lymph nodes with extension to colon, ileum and bladder, bronchopneumonia. Microscopically one kidney showed large calcareous deposits in the collecting tubules.

Suprarenals: Marked deposition of calcium in the reticular layer of one suprarenal. No calcification found in the other. Very dense calcium deposits with a thin connective tissue capsule and trabeculae extending into the calcified areas.

CASE 4. *Clinical History:* J. S. Autopsy No. 4247, female, 41, Russian. Mother died of cancer of the stomach. Measles, mumps, pertussis in childhood. Pleurisy and pneumonia two years before. Patient had cough with blood-streaked sputum at 18 years. Enlarged glands with pruritus for seven years. She developed a right otitis media and terminal sepsis. Blood sugar 100 mg., hemoglobin 68 per cent., blood urea 17 mg. She received very little X-ray therapy on account of low white blood count. She ran a terminal septic fever for six weeks.

Anatomical Findings: Splenomegaly of undetermined origin, extensive bronchopneumonia in right lung, healed tuberculosis of right upper lobe. Microscopically the spleen showed marked proliferation of the reticular and endothelial elements.

Suprarenal: Marked deposition of calcium in the reticular layer of the cortex. similar to Case 3 excepting that calcium deposition was not so marked and there was less connective tissue. Calcium was light staining and present in only one gland.

DISCUSSION

The etiological factor in the formation of this particular lesion has not been satisfactorily established.

It is easy to assume that hemorrhage in the suprarenal with subsequent calcification would account for it. Calcification in areas of hemorrhage has occurred in several reported instances, but calcification is very rare considering the frequency of suprarenal hemorrhage in the newborn. It is possible that an extensive but sublethal hemorrhage, at birth, might go on to calcification in the process of healing. Such deposits might then remain throughout life as in cats. In favor of this view is the localization of the calcification in the reticular layer of the cortex. Against this is the absence of calcification in the medulla.

Calcification as a terminal process of resolution in areas of necrosis such as occurs in tuberculosis, syphilis, and other chronic inflammatory lesions is of common occurrence. Such a lesion does not appear to have been a factor in the cases here reported. The absence of marked connective tissue proliferation and other signs of chronic inflammation would tend to substantiate such an opinion.

An embryonal metaplasia may account for the presence of bone in the cases of Newsam and Kruse. It would be difficult to explain the observation of Wooley in this way, although the tuberculous process might have secondarily involved the suprarenal gland.

Disturbances in the acid-base ratio may lead to calcification in other organs, but it has not yet been produced in the suprarenals in this manner.

A disturbance of function of the glands of internal secretion, particularly the parathyroids and thymus, could be an etiological factor as suggested by Harbitz.

Degenerative changes in the renal epithelium with deposits of lime salts are frequently seen in localized X-ray radiation of these organs (Warthin,[16] Hartman, *et al.* [17]). It is possible that a similar result might occur in the suprarenals. However, in our series, the suprarenal area was irradiated in only one of the cases with one half the erythema dosage. I do not believe that X-ray radiation played any rôle in the calcification of the suprarenals in these cases.

The presence of a specific toxin of bacterial or other origin could cause necrosis with subsequent calcification of the suprarenal. Since distemper is the most important etiological factor in the calcification of the cat's suprarenals it is possible that the somewhat comparable disease (influenza) in man which is known to markedly affect the suprarenals may have been a factor in our human cases. In our series it was not possible to obtain any evidence implicating influenza.

Summary

Four cases of calcification of the reticular layer of the cortex of the suprarenal gland in 1185 autopsies in a hospital for chronic diseases are here reported. Several possible etiological factors are discussed.

I am indebted to Dr. David Marine for his many helpful suggestions.

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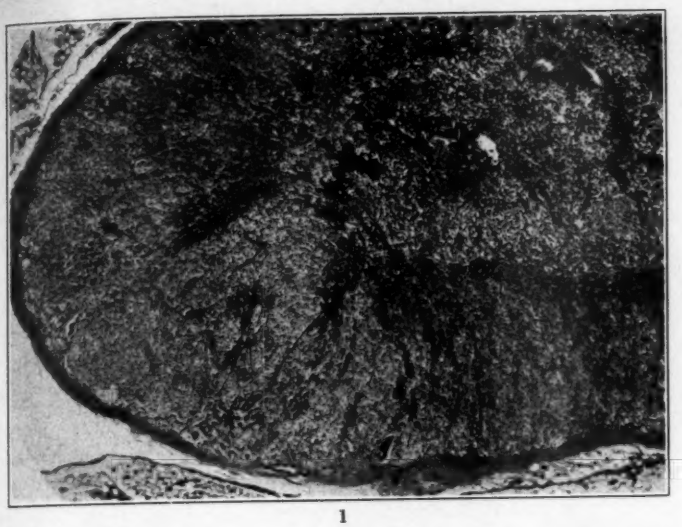
DESCRIPTION OF PLATE

PLATE 103

FIG. 1. Case 1, photomicrograph of a recent area of calcification in the reticular layer of the cortex. × 40.

FIG. 2. Case 3, photomicrograph showing extensive areas of calcification in the reticular layer of the cortex. × 40.

FIG. 3. Case 3, photomicrograph of a small area of Fig. 2 showing crystalline deposits of calcium salts in fascicular and reticular layers of cortex. × 200



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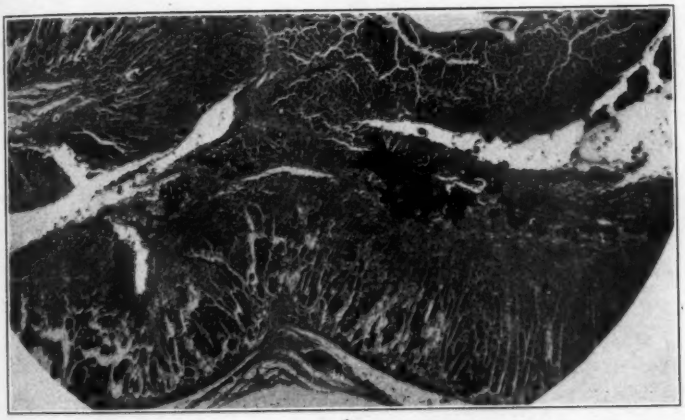

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3

STUDIES ON THE BONES IN AVIAN RICKETS*

I. BONE LESIONS IN CHICKENS DEPRIVED OF THE ANTIRACHITIC FACTOR AFTER FIVE WEEKS OF NORMAL GROWTH

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(From the Department of Anatomy, Cornell University Medical College, New York, N.Y., with the coöperation of Mount Hope Farm, Williamstown, Mass.)

When chicks receiving a ration poor in antirachitic vitamine are deprived of direct sunlight they develop after a few weeks a condition known as "leg weakness." As implied by the name the disease is characterized by marked difficulty in standing and moving, usually accompanied by growth retardation and symptoms of anemia. Morphological changes, some of which are only apparent at late stages of the disease, have been described by several investigators. Disturbances in the processes of ossification manifested in production of abundant osteoid, spontaneous fractures and beading of the ribs have been described by Doyle.[1] The parathyroids appear enlarged (Doyle [2]), and in advanced cases of the disease their parenchyma shows regressive changes (Nonidez and Goodale [3]). Finally, there are also metabolic changes, the most important of which are low phosphorus and calcium content in the blood (Hart *et al.*;[4] Ackerson *et al.*;[5] Steenbock *et al.*;[6] Hughes *et al.*[7]) and in the bones (Hart *et al*[8].). It is not surprising then that leg weakness has been generally regarded as the equivalent of mammalian rickets.

As a result of comparative studies of bone lesions in leg weakness and in experimentally produced low phosphorus rickets in rats, Pappenheimer and Dunn [9] concluded that leg weakness is not rickets. "An excess of osteoid tissue, perhaps the most significant and distinctive feature of rachitic bone, was never present in the chick preparations. Indeed, the amount of osteoid seemed diminished in comparison with that normally found in the healthy growing control of the same age." (*Cf.* p. 722.) The age of the chicks examined ranged from 16 to 37 days. They were placed under experimental conditions soon after hatching.

Hughes and Titus [10] have pointed out that Pappenheimer and Dunn failed to find typical rachitic changes in the skeleton because these investigators examined only very young chicks. They have

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also suggested that the differences between the microscopic bone lesions of leg weakness and experimental low phosphorus rickets in rats may be due to the fact that the same disease produces different histological changes in species as widely diverse as the chicken and the rat.

The present study of the bones of chickens affected with leg weakness at different ages furnishes an explanation of the divergence of opinion that exists on the subject. But, in order to understand properly the effect of lack of the antirachitic factor on the skeleton, its condition at the time of hatching and during the first weeks of life must first be reviewed.

In the newly hatched chick, as shown by several of the early authors and more recently by Fell [11] ossification is little advanced and there is an abundance of embryonic cartilage at the ends of the diaphyses of the long bones. The latter consist of cylinders of firm periosteal bone enclosing the marrow and unreplaced cartilage. At this stage the spongiosa is represented by a few bony trabeculae of endochondral origin placed under the periosteal bone at the diaphyseal ends. The bulk of the spongiosa develops, therefore, after hatching. In the long bones of adult chickens the spongiosa is well developed in the diaphyseal ends but its trabeculae are thinner than the corresponding structures of mammalian bones.

The development of the spongiosa is preceded by the penetration of numerous vessels from the marrow cavity into the almost continuous band of embryonic cartilage. The invading vessels cut the band into a series of irregular strips, more or less parallel to the axis of the bone; the bases of the strips are continuous with the zone of proliferation. The strips just mentioned form the zone of provisional calcification. Bone is deposited around each strip and at first appears as an osteoid margin. This in White Leghorns at least does not reach the bases of the strips until the end of the fourth or the middle of the fifth week after hatching. At this moment most of the cartilage present in the diaphyses is of recent formation in the zone of proliferation, the embryonic cartilage having been replaced by bone, and the bones have only now reached a condition corresponding to that in newly born mammals.

From these considerations it is plain that although to external appearances newly hatched chicks are in a more advanced stage of development than most of the newly born mammals used for labora-

tory work — if we judge from their general activity and the development of their sense organs — their bones are in a condition only paralleled in the mammal during fetal life. *A priori* it is questionable whether comparison of the lesions present in juvenile avian leg weakness with the lesions in experimentally produced mammalian rickets is fair since the treatment begins in each case when the bones are in different stages of development. There are, moreover, other factors that have an important bearing on the skeletal changes observed in early stages of the disorder produced by lack of the antirachitic factor on chickens.

Under the adverse conditions of continuous existence indoors and a deficient diet, growth of the bones and replacement of their cartilage by endochondral bone are retarded in young chicks. The extent of this retardation varied in our experiments according to the diet. Chicks that received a basic ration * not supplemented with semisolid buttermilk were very small at six weeks and few survived after this period. The condition of their bones was very similar to that described by Pappenheimer and Dunn and can be scarcely spoken of as "rachitic." Although the band of embryonic cartilage has been cut into strips by the vessels from the marrow cavity, as happens in normal chicks, the strips lack a well developed osteoid margin. The spongiosa is represented by a few trabeculae; hence the osteoporotic aspect of the bones. When, on the other hand, semisolid buttermilk was added to the diet better growth was obtained but rachitic lesions were not present during the first six weeks of life, although the processes of ossification were more normal than in the chicks not receiving buttermilk. Lesions appeared, however, at a later age, and were very pronounced in two birds kept continuously indoors for four and a half months. Apparently, growth retardation though not so marked as in the case mentioned above prevents the early appearance of such rachitic changes as enlargement of the ends of the long bones, beading of the ribs, and formation of abundant osteoid since the development of these abnormalities depends on active growth.

With these facts in mind it became apparent during the course of the work that the problem of the relation of leg weakness to mam-

* The basic ration used in the experiments was scratch feed containing equal parts of yellow corn, wheat and oats, and a dry mash made up of yellow corn meal, bran, sifted ground oats, white middlings and meat scrap with about 12.5 per cent of bone.

malian rickets could not be satisfactorily solved when newly hatched chicks are used for the experiments. Obviously, from the comparative standpoint, the ideal requirements for a study of the problem are realized when the treatment is applied to chickens previously allowed to grow normally for a period of five weeks. At this time, as already stated, the bones are in a stage of development that closely resembles the condition of mammalian bones during the first weeks of life, and any disturbing influence of the early stages of growth can be easily eliminated.

In the present paper the results of the experiment outlined above will be reported. Thirty-three White Leghorn chicks, hatched May 31, 1926, received exposures to sunlight, when available, during the first five weeks of life. The length of exposure was not uniform but averaged about fifteen minutes daily in the first week; this was increased to about one hour when the chicks were three weeks old. A diet consisting of scratch feed and dry mash was fed.* In order to obtain better growth the diet was supplemented with semisolid buttermilk for four weeks. During the fifth week this was gradually discontinued. At the end of the fifth week four chickens were autopsied and some of their bones preserved for histological study. The other birds were confined in a brooder house, where they could not receive direct sunlight. They were fed the same diet as before, with the exception of the buttermilk, which had been previously withdrawn from the ration.

Twenty-three chickens were autopsied after four to five weeks of existence indoors, while six birds, exhibiting diverse degrees of leg weakness, were placed in a chicken house opening into a yard, where they could receive sunlight at will. The condition of these birds soon improved; two of them were killed after two weeks, the others after six weeks of existence in a normal environment. Their bones were preserved for study.

The writer wishes to acknowledge the spirit of coöperation shown by Mount Hope Farm, Williamstown, Massachusetts, where the experiment was conducted under the direction of Dr. H. D. Goodale, to whom he is also indebted for suggestions and criticism.

* The same basic ration mentioned in previous footnote was used in this experiment.

I. Gross Skeletal Changes

While the combined effect of lack of sunlight and a diet deficient in antirachitic vitamine during the first weeks of life is manifested in decreased growth rate, the growth of chickens receiving the treatment at a later age was little affected and their size was normal for their age. Symptoms of leg weakness began to appear as early as the third week after exposure to unfiltered sunlight was discontinued. In this respect there seems to be little difference between very young chicks and older birds. Various degrees of leg weakness occurred, from an almost complete inability to move about to a nearly normal condition in which the symptoms of the disease were visible only when the chickens tried to jump, or while they were scratching in search of food.

There was, however, a marked contrast when the bones of the chickens in the present experiment were compared with those of chickens deprived of the antirachitic factor shortly after hatching. As already stated the bones of the latter do not show gross rachitic lesions after six or even eight weeks of lack of the antirachitic factor, whereas in the birds of the present study there were deformities similar to those described by Doyle in pullets and laying hens. The ends of the tibiotarsus and femur were slightly enlarged at the close of our experiment in almost all of the twenty-three birds examined. In two males and one female the distal ends of the sternal and vertebral ribs * showed knobs on their inner surface, comparable with the characteristic "beading" of the ribs in the rachitic mammal. Another feature, reported by Doyle, was present in three males and two females; this abnormality consisted in a bending in of the articulation of the two portions of the ribs, which produces a groove in the corresponding thoracic side. In our birds, however, this defect was not bilateral, but was restricted to the left side of the body and in some cases was associated with beading of the ribs. Finally, deformity of the keel of the sternum occurred in five males and one female; all the birds with beaded ribs showed this abnormality. In a general way, judging from the data at hand, it seems that rachitic changes appear earlier in the males; this may be due to the fact that

* The ribs of birds consist of two independent portions held together by fibrous tissue, one articulated with the vertebrae (vertebral ribs) and the other articulated with the sternum (sternal ribs).

the latter grow faster and attain heavier weight when adult than the females.

Spontaneous fracture of the ribs, observed by Doyle in mature chickens, was not present in any of the birds of the experiment described in this paper.

A noteworthy feature of leg weakness in the domestic fowl is the absence of bending of the bones of the legs. At least, bent bones were observed in only one series of the several experiments conducted at Mount Hope Farm and then were not restricted to the chickens deprived af the antirachitic factor.*

Gross frontal sections of the tibiotarsus and femur showed the presence of a transverse band of pink color, situated under the zone of proliferation. This band (Fig. 1, *r*) represents the bone formed during the experimental period and owes its light color to the absence of normal marrow. Its depth varied a good deal in the seventeen chickens examined, but it was never absent. The bone was so soft at this level that it could be cut with the scalpel. Bones of normal chickens lack the band just described since the marrow is well developed throughout.

II. Microscopic Changes

The femur, tibiotarsus and, in a few cases, the articulation of the sternal and vertebral ribs, were preserved and decalcified in picronitric acid for twenty-four to thirty hours. They were washed in running water and embedded in parlodion. Sections were stained with Mann's acid hematein (with or without counterstain) and Rio Hortega's silver carbonate method. The latter gave good results for the study of the finer structure of the osteoid and the reticulum of the marrow, especially when the sections were toned with gold chloride. But, perhaps due to the method of preservation, the cellular contents of the marrow, cartilage and bony trabeculae were not impregnated. When desired, these cells were brought out through counterstaining with indigo carmine.

* In the first experiment bent leg bones occurred in chickens deprived of the antirachitic factor and also in chickens exposed to direct sunlight, irradiated with a mercury-vapor lamp or fed the basic ration supplemented with cod liver oil. As suggested by Goodale [13] the condition of their bones may have nothing to do with lack of the antirachitic factor.

Examined under the microscope, the transverse band of bone formed during the period of existence indoors is seen to consist of broad osteoid trabeculae. The trabeculae are richly anastomosed and may show remnants of unreplaced cartilage. The excess of osteoid in the rachitic band can be well appreciated through comparison of similar regions of the same bone in normal and rachitic chickens. Since the chickens examined were deprived of the antirachitic factor for only four to five weeks, enlargement of the ends of the long bones was not very marked. The depth of the zone of columns of flattened cells and of the zone of hypertrophied cartilage cells (zone of provisional calcification) is very unequal in some specimens suggesting that the process of cartilage replacement by bone was not proceeding at a normal rate. In beaded ribs the bony part is composed of unusually thick trabeculae of osteoid, surrounded by a well defined and, in some places, thickened osteoblastic layer. The zones of proliferation and provisional calcification appear distorted and, in one particular case, replacement of cartilage by bone has taken place in such an irregular fashion that the original band of cartilage has been disrupted and now consists of a distal portion and two lateral masses of unreplaced cartilage, situated more posteriorly (Fig. 2, c', c''). Growth of this displaced cartilage apparently causes the beading so characteristic of rachitic ribs.

The epiphyseal cartilage in these rachitic bones is moderately calcified. The areas of calcification occur in the center of the cartilage, except in the immediate vicinity of the epiphyseal vessels. The presence of calcium in the cartilage under consideration, emphasized by Pappenheimer and Dunn in young chicks, can be accounted for when we keep in mind that since in the fowl there are not independent epiphyseal ossifications,* the necessary mechanism for erosion and removal of preëxisting cartilage seems to be absent during the phase of active growth. Ossification of the epiphyseal ends in the long bones of birds takes place when the bones cease growing. Inactivity in the zone of proliferation soon results in its invasion by vessels and osteoblasts, which enter the epiphyseal cartilage from the diaphysis and destroy the calcified matrix, after

* In the epiphyseal cartilage covering the lower end of the tibia there are, however, two independent centers of ossification. These centers are not true epiphyses but represent tarsal elements fused with the tibia; hence the name "tibiotarsus" applied to the latter in most of the treatises on comparative anatomy.

which bone is deposited. It seems likely, then, that the cartilage under discussion became calcified during the first weeks of life and that it remained as such.

Periosteal ossification is very active at the level of the diaphyseal ends. The osteoblastic layer of the periosteum is very thick in this region (Fig. 3, *ol*) as compared with the corresponding layer in normal bones. Evidences of increased bone formation at this level are manifested in rachitic bones in the presence of thick trabeculae more or less at right angles to the surface of the bone. These trabeculae (Fig. 3, *o*) show different shades of pink in slides stained with hematein and eosin. Toward the center of the shaft they are deeply stained and possess small, irregularly shaped osteocytes, while toward the periphery of the bone the trabeculae are much paler and show larger cells; in this region they gradually fade into the osteoblastic layer of the periosteum. At the diaphyseal ends, periosteal bone, in both normal and rachitic chickens, contains stout collagen fibers from the capsule and ligaments of the joint. These fibers are best seen in slides stained with silver carbonate.

Since the use of silver carbonate has revealed interesting features in both the osteoid and the marrow it seemed convenient to describe them in detail. Whether some of the abnormalities observed are restricted to the fowl or whether they also occur in mammalian rickets I am unable to say, inasmuch as the method is comparatively new and, as far as I am aware, has not been used in the histopathology of rickets.

Formation and Characteristics of the Osteoid in the Rachitic Band: Rio Hortega's silver impregnation method stains collagen fibers in the bone matrix (decussating fibers of Sharpey of the mammalian anatomy) and, at the same time, impregnates the fibers of the externa of the vessels and the reticulum of the marrow. The latter appears stained deep purple or black in sections toned with gold chloride while collagen fibers stain light purple.

Some of the collagen fibers of the externa of the vessels are deflected and run freely in the marrow spaces where they act as the main support for the delicate network of the reticulum. In some areas they seem to be continuous with the osteoid fibers.

In common with the bones of rachitic mammals the bones in avian rickets show a thick osteoblastic layer, several cells deep in many places. The osteoblasts are closely packed and the continuity

of the layer is interrupted only by the presence of multinucleated giant cells (osteoclasts). For the most part the osteoblasts have round or oval eccentric nuclei, and their size is variable, from a fairly small cell to abnormally large elements. These overgrown or hypertrophied cells possess relatively small, deeply staining nuclei and are incorporated into the bone matrix (Figs. 8 and 10, *o'*). Large osteoblasts were not found in the bones of those chickens autopsied before the experiment was begun. Furthermore, in rachitic birds they are absent in those portions of bone which were laid down prior to the experiment. The presence of hypertrophied osteoblasts seems, therefore, closely associated with the formation of abundant soft osteoid, and to be one more item due to lack of the antirachitic factor.

The structure of the matrix differs somewhat in bone trabeculae deposited before and during the experiment. In the former the fibers are thin and parallel; the outlines of individual fibers are often difficult to see since they are cemented together by an interfibrillar substance in which calcium salts are presumably deposited. In rachitic areas the fibers are of irregular thickness and in some places they exhibit a tendency to form definite bundles. Furthermore, in many areas they are not parallel but run in different directions. The histological picture suggests a disorderly arrangement of the fibrous material (Fig. 4, *o*).

In normal bone recently formed osteoid around the prolongations of cartilage emanating from the zone of proliferation appears under the aspect of a well defined osteoid margin (Fig. 7, *om*). The osteoid margin is also present in rachitic bones, but it is usually somewhat thicker (Fig. 8, *om*). In normal bone the osteoid margin gradually increases in thickness, but the osteoblasts never penetrate the calcified cartilage of the prolongations that constitute the zone of preliminary calcification. In sections stained with silver carbonate the fibers run parallel and they are closely applied against the surface of the cartilage (Fig. 5). Here and there, however, one or several lacunae of the cartilage have been partly corroded and osteoblasts are seen in their interior, together with much bent fibers which fill the lacunae and give the impression of a complicated intralacunar network.

In rachitic bone, on the other hand, the osteoblasts freely enter the cartilage of the prolongations mentioned above and form fibers

running in every direction. In this way a very coarse network of fibers enclosing cartilage cells is produced (Fig. 6). The penetration of osteoblasts and the formation of intracartilaginous fibers are probably favored by the absence of calcification of the cartilage.

In certain areas within the osteoid trabeculae there are groups of hypertrophied osteoblasts. Large multinucleated cells closely resembling the osteoclasts also occur within the trabeculae. It was thought at first that such cells might be true osteoclasts which had penetrated the trabeculae and that the absence of any erosion suggesting the path of their entrance into the osteoid could be explained on account of the plane of the section. Repeated observations, however, indicated that in no case was there communication with the intertrabecular spaces. Finally, it was clear that intra-osteal multinucleated cells arise *in situ* as a result of migration and coalescence of hypertrophied osteoblasts. An early stage of their formation has been represented in Fig. 9. In all probability lack of calcified cementing substance between the osteoid fibers enables the osteoblasts to glide along the interfibrillar spaces and gather in groups. It cannot be denied, however, that the multinucleated cells may arise as the result of a combined process of division and coalescence of free osteoblasts, but mitotic or amitotic processes, suggesting that cell division plays an important part, were never found in the slides. Absence of corroded areas of osteoid around the large multinucleated cells strongly suggests that these elements are not active bone destroyers, and in spite of their similarity to the osteoclasts they are probably a different type of cell arising as a result of the osteoblastic hyperplasia prevalent in the rachitic band.

Changes in the Marrow: Lack of the antirachitic factor prevents the development of normal marrow during the experimental period but does not cause degenerative changes in the marrow already present in the bones at the beginning of the experiment. On the contrary, instead of degeneration there is a tendency toward hyperplasia of the fully differentiated marrow. Hyperplastic processes are not general, but restricted to small foci that appear as nodules made up of much crowded cells. In slides stained with hematein-eosin the nodules somewhat resemble lymph nodes, known to occur in the marrow in some cases of human rickets (Schridde [13]). Close study, however, shows that lymphocytes are absent and that many cells contain eosinophilic granulations. Undoubtedly, the

nodules under discussion are the source of cells of the leucoblastic and erythroblastic lines. They occur chiefly in the vicinity of the rachitic band. It does not seem unlikely that hyperplasia in this case is of a compensatory character, inasmuch as blood-forming elements are very scarce in the marrow of the rachitic band.

The aspect of the marrow in the rachitic band is very similar to that of the corresponding structure in rachitic mammalian bones. The spaces between bony trabeculae are occupied by large numbers of loosely arranged, spindle-shaped cells, oriented in every direction save in the proximity of the trabeculae where the predominant direction is parallel to the trabecular surface (Fig. 10). A certain amount of fibrous tissue also exists around those trabeculae of the spongiosa deposited prior to the experiment, and also on the inner surface of the cortex. The atypical marrow in these locations has apparently taken the place of bone reabsorbed during the process of enlargement of the central cavity of the bone. Since the bony trabeculae in chickens are more slender and not so numerous as in mammalian bones the intertrabecular spaces appear larger than the corresponding spaces of the mammal, even though there has been marked thickening of the trabeculae formed during the experimental period.

The marrow within the rachitic band does not contain fat cells, and myelogenous elements form discrete groups in which a few myeloblasts and myelocytes are recognizable. The spaces between the spindle-shaped cells seem to be filled with a watery fluid. Lack of myelogenous tissue causes the pink color of the rachitic band in sections of fresh bones, as compared with the red color in normal bones.

Structural differences between normal and rachitic marrow are further illustrated by slides stained with silver carbonate and toned with gold chloride. Figs. 11 and 12 represent normal and rachitic marrow, respectively, from the same section. For the sake of clearness the faint cell outlines have not been indicated. The normal marrow at the right of the capillary (*bv*), in Fig. 11, contains fat cells; at the left it is much more compact. This compact area is a hyperplastic nodule. It will be noticed that the reticulum in the latter is very well developed, while in the region with fat cells reticular fibers are not so abundant. Collagen fibers (*co*), arising from the externa of the vessels, form the main support of the mesh-work.

The outstanding fact in the marrow within the rachitic band is the condition of the reticulum (Fig. 12, *r*). In order to appreciate fully this difference it must be stated that both figures in the Plate are camera lucida drawings made under the same magnification. Photographs were not made since the meshes of the reticulum would not be clearly seen in a single plane. Fine fibrils, such as are seen in normal marrow, are absent or poorly developed. Instead, there is a meshwork of faintly stained, coarse trabeculae. In hematein-eosin slides the reticulum is not clearly visible but its thicker portions are seen as indistinct pink masses suggesting colloid. At first it was thought that these masses were due to degenerative changes in the marrow, but the application of silver carbonate soon revealed their true nature.

The abnormal condition of the reticulum within the rachitic band was at first regarded as the result of swelling of its fibers. In a recent paper Mallory and Parker [14] have shown that separated collagen fibrils are deeply stained by silver, but compacted fibrils are not. According to these authors reticulum is merely collagen occurring as separated fibrils; when "the fibrils of the reticulum are brought into close apposition through degeneration and disappearance of intervening cells, they no longer stain like reticulum but like collagen." The fact that the marrow in the rachitic band fails to develop normally and that it has relatively few cells strongly suggests that the pale color of the coarse fibers of the reticulum in this area might also be the result of coalescence of collagen fibrils. In order to test this point I stained with anilin blue some sections already stained with silver carbonate. This technique has revealed that the collagen fibers of the normal marrow (Fig. 11, *co*) and the supposedly swollen reticulum take a brilliant blue stain: furthermore, the "swollen" trabeculae of the reticulum are seen to consist of closely packed fibrils. From these facts it seems clear that although absorption of fluid from the marrow spaces may cause a certain amount of swelling in the reticulum this process is far less important than the apposition of considerable numbers of collagen fibrils; and that when this apposition takes place, as pointed out by Mallory and Parker, the compacted fibrils no longer stain like reticulum but like collagen.

The walls of the vessels in rachitic marrow show certain abnormalities that deserve mention. In growing long bones the diaphyseal ends contain slender blood vessels arising from the vascular

network in the marrow of the central cavity of the bone. The vessels run parallel to the axis of the bone, and a number of them reach and cross the zone of proliferation and enter the epiphyseal cartilage where they anastomose with the epiphyseal vessels. The latter enter the cartilage chiefly from its posterior surface and they are already present at hatching time (Fell). The walls of the slender diaphyseal vessels in normal chickens consist of endothelium and an outer adventitial layer formed by delicate collagen fibers enclosing spindle-shaped cells with prominent nuclei.

In the rachitic band the walls of the vessels just described are very thick (Fig. 10, *v*). In hematein-eosin slides the adventitial cells stand out sharply in the midst of the thickened walls. In sections stained with silver carbonate it is easy to see that thickening of the walls of the vessels is due to increase and possibly too to swelling of the adventitial fibers, many of which leave the vessel to enter the reticulum (Fig. 12).

The capillaries of the rachitic marrow are well developed; changes in their walls, similar to those found in larger vessels, are absent. A normal amount of blood is seen in the capillaries.

The condition of the marrow in the rachitic band indicates the presence of an excess of watery fluid in the intertrabecular spaces. This excess has been shown to exist by several of the early pathologists and more recently by Schabad, and Korentchevsky.[15] In addition to the histological aspect of the rachitic marrow there are in some cases other evidences of excessive amount of fluid. Large, irregular cavities are often seen in both the rachitic and normal marrow of rachitic bones; when very large these spaces are visible to the naked eye in gross sections of the bones (Figs. 1 and 2, *l*). Under the microscope the cavities under discussion usually appear empty, through loss of the fluid contained therein after the bones were split for preservation, but there are instances in which they contain a delicate network with a few imprisoned lymphocytes and leucocytes.

The cavities mentioned above possess a definite lining consisting of much flattened cells. My impression is that they are considerably distended lymphatics. The latter are well developed throughout the marrow in the fowl, where they form a loose network with sinusoidal aspect. From this network there arise slender vessels that travel toward the epiphyseal ends of the bone and end blindly be-

tween the strips of cartilage of the zone of provisional calcification. In sections of normal bones the lymph vessels are filled with coagulated plasma containing a few lymphocytes and an occasional leucocyte. In rachitic bones many of the marrow lymphatics are absent, but their place is taken by the large cavities which on account of their distension with fluid cause a characteristic condensation of the osteoblastic layer around the prolongations of cartilage in the zone of provisional calcification (Fig. 8).

In the light of present knowledge on the function of the lymph vessels we may assume that the excess of fluid accumulated in the intercellular spaces of the marrow within the rachitic band is drained by the lymphatics. The wide cavities present in the normal marrow of rachitic chickens represent the larger trunks of the system distended with fluid collected by the branches within the rachitic band.

Comment

The descriptions in the preceding pages point to the conclusion that when the antirachitic factor is withheld from partly grown chickens a disorder develops with all the characteristics, both gross and microscopic, of mammalian rickets. Minor differences in the aspect of the avian lesions are undoubtedly due to underlying differences in bone development and structure. In the experiment reported in the present contribution rachitic lesions developed readily but this is probably due to the fact that the chickens had received only enough exposure to sunlight to obtain normal growth; if the birds had been continuously outdoors before the experiment was begun, rachitic changes in the bones might not have been so marked at the end of four weeks.

I quite agree with Pappenheimer and Dunn that leg weakness in chickens deprived of the antirachitic factor during the first weeks of life is not rickets if we regard the characteristic lesions in the bones as the chief diagnostic feature of the disease. As will be shown in another paper rachitic lesions develop very slowly when newly hatched chickens are reared in the absence of direct sunlight, and they are not quite as typical as when the experimental treatment is applied to chickens reared in a normal environment for four or five weeks. But even though the lesions in very young chickens are not clearly of the rachitic type it does not seem advisable to deny alto-

gether the existence of rickets in these birds since rickets is not a disease of the bones but a disorder of nutrition that eventually affects the bones. Furthermore, the evidence gathered from humans shows that rickets is more apt to occur during the second half of the first year and first half of the second year. The absence of rachitic changes in the bones of chicks during the first weeks of life cannot, therefore, be used as a strong argument against the identity of leg weakness with mammalian rickets. On the contrary, it would rather indicate that the causative factors operate in a similar way in animals as different as the mammal and the bird.

In concluding I wish to say that although the formation of abundant osteoid and deficient deposition of phosphorus and calcium in the bones have been repeatedly emphasized by most authors as the outstanding characteristics of rickets, conditions in chickens have revealed other features that may have an important bearing on the production of rachitic lesions. The excessive amount of fluid in the marrow in the rachitic band and distended condition of the lymphatics suggest changes in the permeability of the walls of the growing vessels. It would not be surprising, then, if the deficiency in phosphorous and calcium deposition were due to an altered physicochemical equilibrium that prevents or hinders passage of one or both of these elements through the young capillary walls, imperfectly developed under the stress of a general disorder of nutrition. In the event of its existence such a mechanism would afford a certain degree of protection inasmuch as it would tend to prevent depletion of the already low blood phosphorus which, although needed for normal growth of the skeleton, is also essential for the fulfilment of other physiological activities. While in mammalian rickets there is no deficiency in the calcium content of the blood, in chickens calcium is low, a fact that may account for the marked enlargement of the parathyroids in birds deprived of the antirachitic factor (Doyle, Nonidez and Goodale).

Summary

1. Lack of the antirachitic factor in chickens previously allowed to grow normally for a period of five weeks leads to a disorder with all the essential characteristics of mammalian rickets.

2. Gross skeletal changes, such as beading and bending of the ribs, deformity of the keel of the sternum and enlargement of the

epiphyseal ends of the long bones were present in several chickens within five weeks after the beginning of the experiment.

3. The bone formed during the experiment appeared under the form of a band placed in long bones between the epiphyseal cartilage and the diaphysis.

4. Microscopic lesions observed in the rachitic band were: (1) excess of osteoid in the spongiosa; (2) active production of periosteal osteoid; (3) increase in thickness of the osteoblastic layer throughout the bone of the rachitic band; (4) abundance of osteoclasts; (5) absence of calcification in the zone of provisional calcification.

5. The marrow of the rachitic band was found to consist chiefly of spindle-shaped cells separated by abundant intercellular fluid, with small groups of myelogenous cells. Capillaries were abundant. That portion of the marrow present in the bones before the beginning of the experiment was practically unaltered, but a few hyperplastic nodules with crowded myelogenous cells were noticed in the vicinity of the rachitic band.

6. Certain peculiarities are described in detail. The most important are: (1) deposition of osteoid fibers without previous erosion of cartilage; (2) intra-osteal formation of giant cells through fusion of hypertrophied osteoblasts; (3) enormous increase in thickness of the reticulum in the marrow of the rachitic band with a corresponding thickening of the walls of the blood vessels; (4) marked distension of the lymphatics.

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DESCRIPTION OF PLATES

PLATE 104

FIG. 1. Frontal section of the upper end of the tibiotarsus (No. 270, ♂). (*e*) epiphyseal cartilage; (*h*) zone of preparatory calcification; (*l*) lymphatic spaces; (*me*) endochondral bone with normal marrow; (*p*) zone of proliferation; (*r*) rachitic band. × 2.

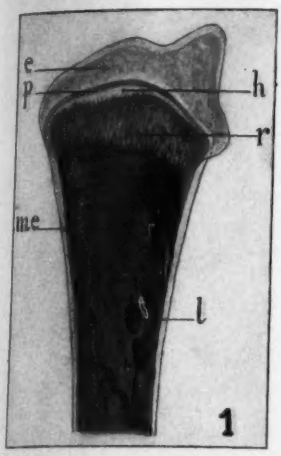
FIG. 2. Longitudinal section through the enlargement of a vertebral rib (No. 255, ♂). Hematein-eosin: (*c'*), (*c''*), lateral masses of cartilage; (*e*) epiphyseal cartilage; (*l*) distended lymphatics; (*o*) periosteal osteoid; (*p*) zone of proliferation of the diaphyseal cartilage. × 17.

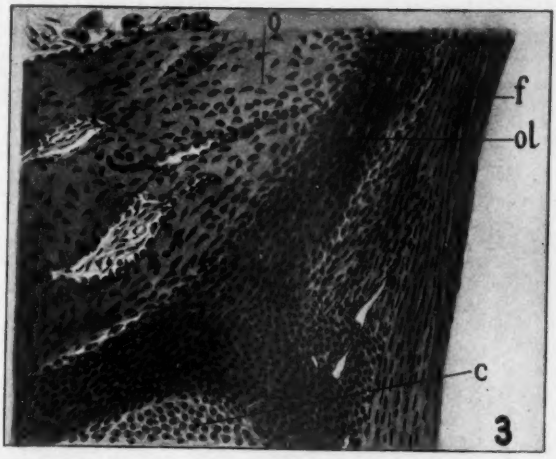
FIG. 3. Active periosteal ossification. The area represented is marked (*o*) in the preceding figure. (*c*) uncalcified cartilage of the lateral mass of cartilage (*c'*) of the preceding figure; (*f*) fibrous layer of the periosteum; (*o*) young osteoid; (*ol*) thickened osteoblastic layer of the periosteum. Hematein-eosin. × 107.

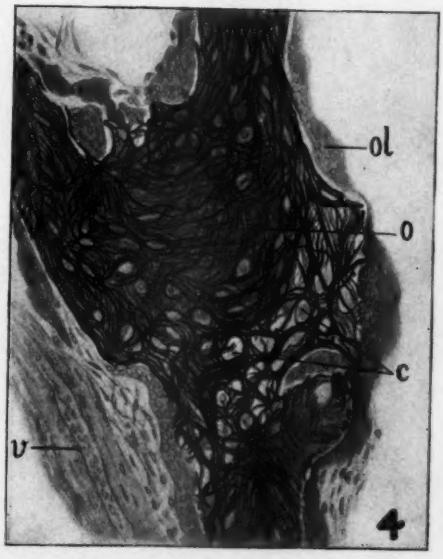
FIG. 4. Trabecula of young osteoid (*o*) in the tibiotarsus. Silver carbonate method; (*c*) enclosed cartilage cells; (*ol*) osteoblastic layer; (*v*) blood vessel with thickened walls. × 266.

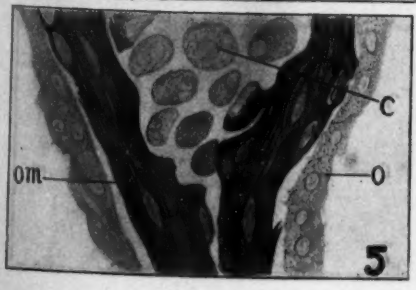
FIG. 5. Normal femur (No. 254, ♀). osteoid margin (*om*) at the distal tip of one of the strips of cartilage of the zone of provisional calcification. Silver carbonate, gold chloride; (*c*) cartilage (unstained); (*o*) osteoblastic layer. × 532.

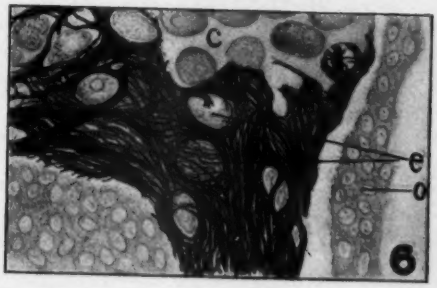
FIG. 6. Rachitic femur (No. 256 ♀). Tip of a strip of cartilage of the zone corresponding to the zone of provisional calcification of the normal bone. Cartilage matrix invaded by osteoid fibers; (*c*) uninvaded cartilage; (*e*) enclosed cartilage cells; (*o*) osteoblastic layer. Silver carbonate gold chloride. × 532.











Nonidez

Studies on Bones in Avian Rickets

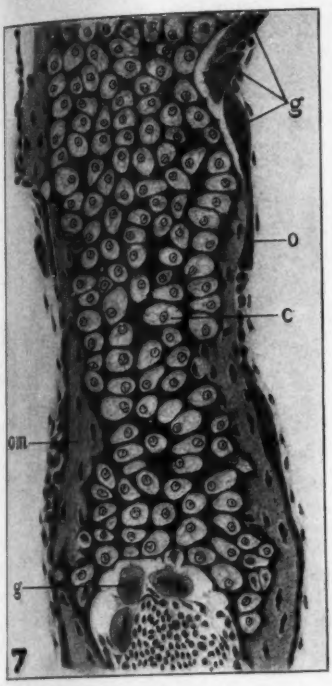
PLATE 105

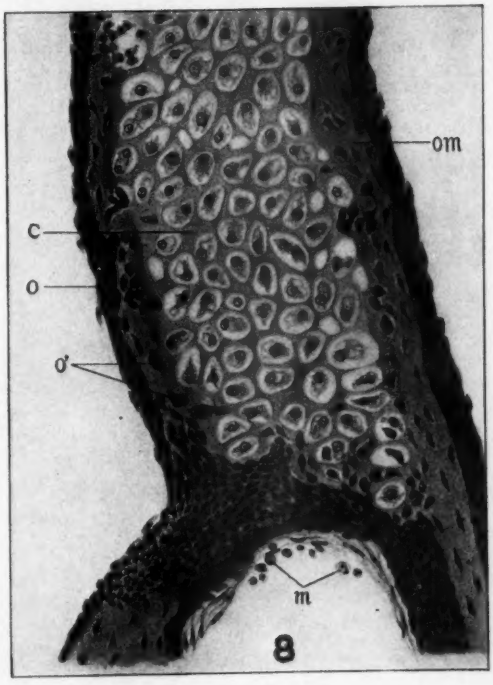
FIG. 7. Strip of calcified cartilage from the zone of provisional calcification of the tibiotarsus of a normal chicken (No. 253, ♀). Hematein-eosin; (*c*) cartilage cells; (*g*) giant cells (osteoclasts); (*o*) osteoblastic layer; (*om*) osteoid margin. × 266.

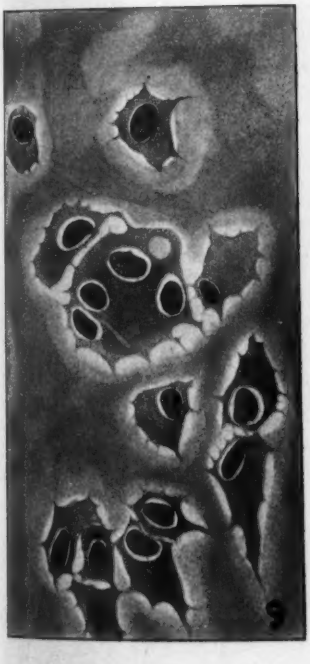
FIG. 8. Strip of uncalcified cartilage from the same zone of the tibiotarsus of a rachitic chicken (No. 255, ♂). Hematein-eosin; (*m*) marrow cells (myelocytes); (*o'*) hypertrophied osteoblasts. Other letters as in the preceding figure. × 266.

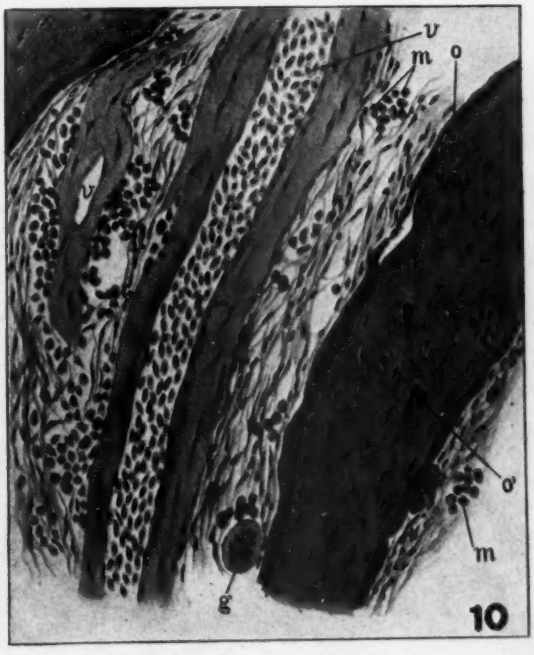
FIG. 9. Hypertrophied osteoblasts in early stages of their fusion to form intraosteal multinucleated cells. Hematein-eosin. × 666.

FIG. 10. Marrow and vessels of the rachitic band of the tibiotarsus (No. 256, ♀) Hematein-eosin; (*g*) osteoclast; (*o*) osteoblastic layer; (*o'*) hypertrophied osteoblasts; (*m*) myelogenous cells; (*v*) diaphyseal vessel. × 266.









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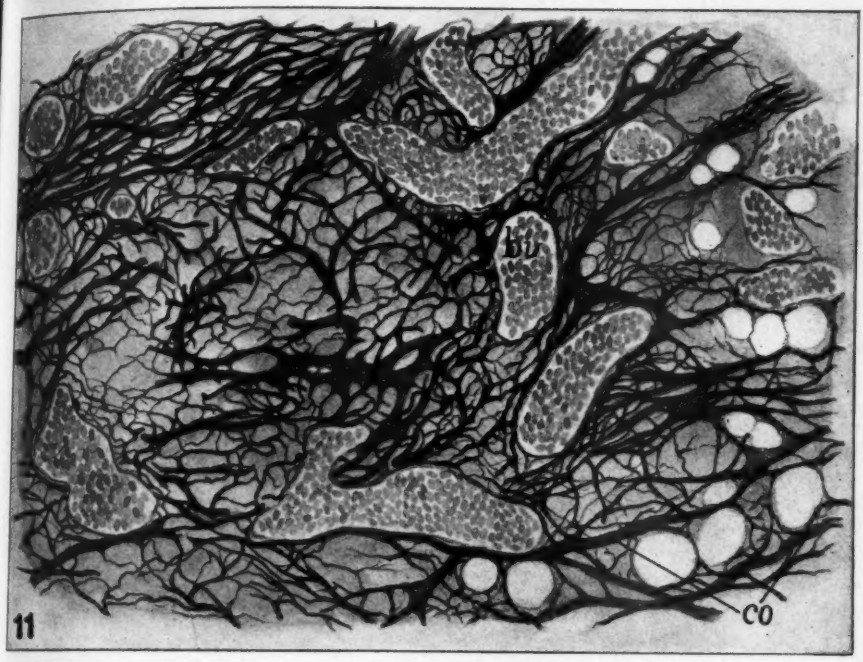
Studies on Bones in Avian Rickets

PLATE 106

FIG. 11. Reticulum of normal portion of marrow in the femur of a rachitic chicken (No. 256, ♀). Silver carbonate, toned with gold chloride. (*bv*) blood vessel; (*co*) collagen fibers. × 266.

FIG. 12. Thickened reticulum (*r*) in the rachitic band. Same section and technique as in the preceding figure. (*bv*) longitudinal diaphyseal vessel; (*cv*) capillaries; (*m*) marrow spaces; (*oi*) osteoid trabeculae. × 266.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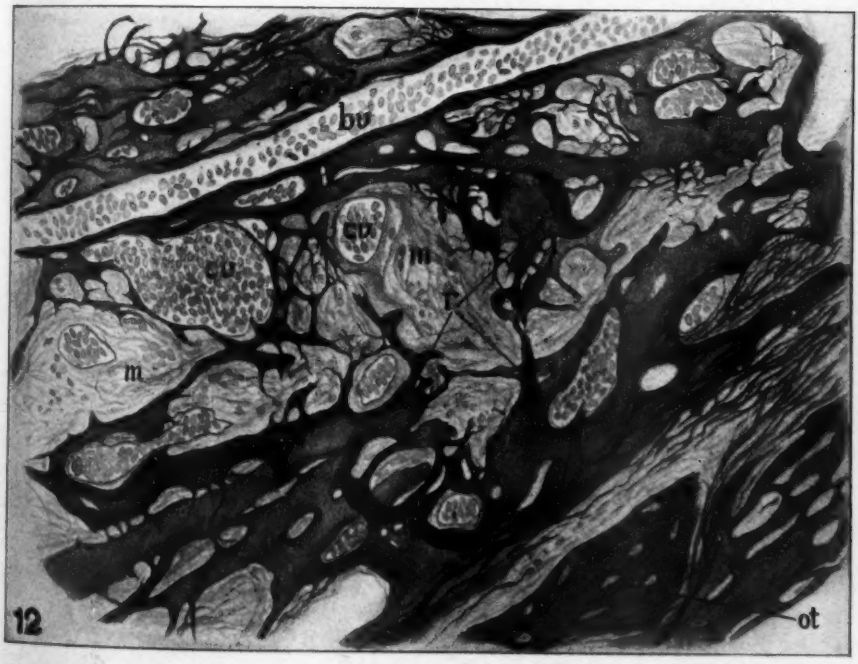
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ABERRANT THYROID GLANDS*

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A review of the literature of thyroid pathology brings out the fact that cases of lateral aberrant thyroid glands are apparently increasing. At the present writing about forty-five cases have been collected from the literature. While the number of cases presented thus far is still comparatively small, we are convinced that the condition is much more common than these reports would suggest. It is probable that there are many other cases which have not been reported, and still others which have been inaccurately diagnosed, as routine histological examinations are not carried out in all hospitals. It is the exceptional case which is diagnosed preoperatively, and, as in our cases, the diagnosis is usually made by the pathologist. The clinical diagnosis is usually "tuberculous glands," "Hodgkin's disease," "lymphosarcoma," "metastatic carcinoma," etc., and without an histological examination such inaccuracies are apt to persist. It is probably the greater tendency for routine pathological examinations of surgical material which accounts for the apparent increase in these cases, as no other obvious factors are to be noted.

It is important to consider this interesting condition, both clinically and pathologically, for several reasons; first, because the developmental side of the question is much discussed and far from being settled; second, because its frequent occurrence and pathology establish it as a clinical entity of note; third, its tendency toward malignant degeneration becomes of serious import to the patient; and fourth, because these glands may be greatly influenced by the changes in the normal and pathological thyroid gland itself.

EMBRYOLOGY AND COMPARATIVE ANATOMY

The development of the thyroid gland is one of the most disputed problems embryologically. It was felt that a study of these anomalously placed bits of thyroid tissue might be of some value in its solution.

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The older embryologists (His, Born, Prenant and others) concluded that the thyroid has a dual origin. They believed that the major portion of the gland, including the isthmus, is derived from a median down-growth of epithelium from the floor of the pharynx. This occasionally persists as the thyroglossal duct. The remainder of the gland they considered as arising from the lateral outpocketings of the pharynx which normally fused with the median portion. On this basis it would be simple to explain the presence of aberrant glands as due to a failure of the lateral portion to fuse with the median.

We have since come to accept the fact that in man the third and fourth branchial clefts give rise to small masses of epithelial cells which migrate toward the thyroid and eventually come to rest, usually at its anterior and posterior borders. These are the parathyroid glands, which in postnatal life are usually cords of cells, but may develop lumina in which accumulates a pale staining colloid-like material. This phase of parathyroid physiology is one which has not perhaps been generally recognized and has given rise to some of the confusion concerning the aberrant thyroid glands, as they have many points of similarity under the microscope. The parathyroids in man vary considerably in both number and position, but are usually paired and four in number. It may be said that anatomists today are generally in accord regarding these two branchial pouches.

Following the discovery of the parathyroid glands, it was believed that the thyroid was developed entirely from the median anlage. Subsequent investigation, however, revealed a developmental relationship between the "fifth" pouch and the thyroid gland. The textbooks give opposing views on this subject and we find little agreement concerning the existence of such a pouch.

Kingsbury [1] studied a series of embryos and came to the conclusion that there is no reason for considering that the ultimobranchial body represents any specific fifth pouch, but is merely formed by continued growth activity of the posterior portion of the pharyngeal complex. In the mechanics of growth, by the shiftings that occur, this posterior portion becomes fused with the thyroid gland. He also concludes that "it soon, however, becomes indistinguishable and apparently finally in man disappears (typically) without a trace."

The work of Badertscher [2,3] confirms Kingsbury's investigation and goes a step further. He studied a large series of pig embryos in

all stages of development from the 15 mm. pig to the 270 mm. pig (full term). Then he studied pigs that were a few hours old, one 7½ days old, one 15 days old, one 28 days old, one 42 days old, one 56 days old and in addition several adults. He was able to identify and follow the development and fate of the ultimobranchial bodies through the entire series. He finds that these bodies participate in the formation of the thyroid gland. He states that, "In conclusion, it can be said that since the ultimobranchial bodies fuse with the thyroid gland and also form colloid, the boundary between these structures and the gland becomes obliterated, so that it is impossible to determine the exact relative proportion that is contributed to the thyroid gland by the ultimobranchial bodies and the median thyroid anlage. Owing to the variable developmental behavior of the ultimobranchial bodies, the relative proportion they contribute to the thyroid gland undoubtedly varies in different pigs. It is, however, quite evident that only a relatively small portion of the gland is derived from the ultimobranchial bodies."

Grosser in Keibel and Mall's textbook [4] in referring to the epithelial bodies which develop from these various pharyngeal outpocketings calls attention to the fact that there is considerable variation in development and striking anomalies of position as well as diminution and increase in numbers. It would seem possible then to account for the presence of these lateral aberrant glands as arising from cells in the posterior portion of the pharynx which in migration have failed to fuse with the median thyroid anlage in its descent. In accordance with Virchow's theory of "fetal rests" these cells may subsequently give rise to tumor formation, when they are derived from third or fourth pouch cells, to the so-called "parastruma" and from the fifth pouch to the true aberrant thyroid. The relatively constant type of degenerative lesion is believed to be more than accidental and suggests that these aberrant thyroid glands may have such an embryological origin.

In a series of cases collected from the literature by Billings and Paul [5] they note that 70 per cent with a diagnosis of neoplasm had tumors of the papillary type. To that list is added our series of four cases, all with the diagnosis of papillary cystadenoma or papillary adenocarcinoma.

Neoplasm of median or lingual thyroid is very rare. We have not seen a single case, and can agree with these authors "that there is an

inherent difference in the lateral thyroids as opposed to the median in their tendency toward a specific type of tumor formation." They also recall the dual nature of the pancreas and note that while carcinoma of the acini is not uncommon, carcinoma of the islands is comparatively rare. This is an interesting analogy.

That the thyroid in its development is constantly associated with the migration and fusion of epithelial cells derived from the so-called fifth pouch or posterior outpocketing of the pharyngeal complex (the ultimobranchial body); that these epithelial masses may vary considerably in number and position; that the lateral aberrant thyroid glands tend in a high percentage of cases to undergo papilliferous and cystic degenerative change, a lesion never associated with lingual thyroid tissue; these facts, it would seem, serve to identify these tumors as a specific group, and to account for their origin on sound embryologic evidence.

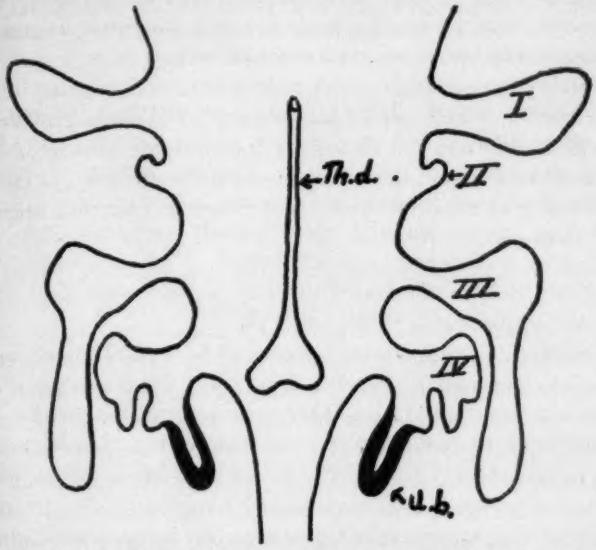
Clinical Discussion

In a series of nearly four thousand cases of thyroid disease seen in the Lahey Clinic only four with lateral aberrant thyroid tumors were encountered, an incidence of only 0.1 per cent. These patients came, not because of thyroid disease, but were alarmed by a slowly growing tumor in the side of the neck. These tumors varied in size, shape and position. They were firm, non-fluctuant, non-tender and gave no subjective symptoms. The duration was from several months to several years. They occurred either as a large single mass or as a chain of gland-like enlargements in the neck along the course of the internal jugular vein. The single type has been as a rule, in our experience, low in the neck, posterior and lateral to the sternomastoid muscle. There are no clinical features other than the location which might aid in diagnosis, and in our four cases the diagnosis has only been suspected once. The importance to the patient, because of the prognosis as compared to that of Hodgkin's disease, for example, cannot be overemphasized, and its consideration in differential diagnosis in lateral tumors of the neck should not be overlooked.

Surgically these tumors should be treated by as complete removal as is possible in each case. It is unnecessary to emphasize the obvious fact that early removal of all suspicious masses in the neck should be strongly urged. No new or special problems of surgical technique

have been encountered in these cases. All have made good recoveries from their operations and thus far no case has had any evidence of recurrence of the disease. The time elapsed since operation has of course been very brief in two of the cases.

We believe that X-ray treatment should be advised when papillary cystadenoma has been found at operation. This type of thyroid



TEXT-FIG. 1. Schematic drawing of the pharyngeal pouches of an early human embryo. I, II, III, IV, lateral outpocketings. *Th. d.*, thyroglossal duct. *U. b.*, ultimobranchial body—posterior continuation of pharynx (Groschuff and Kohn in "Keibel and Mall").

tumor responds most satisfactorily to X-ray treatment and we favor the use of X-ray after operation whether or not the tumor appears to be malignant microscopically. We likewise believe that postoperative X-ray treatment is advisable when multiple areas of aberrant thyroid tissue growth are encountered because of the possibility that malignant changes may already have occurred.

CASE REPORTS

CASE 1. *Clinical History:* N. E. D. H. 30,755. Mrs. B. H., aged 70, entered the hospital July 7, 1925. The chief complaint was a mass in the left side of the neck of 3 years duration. The family and past history are unessential.

Present Illness: Began 3 years ago, when the patient noticed a mass the size of a hen's egg in the supraclavicular space. This has continued to grow upward and posteriorly. It has never been painful or inflamed. X-ray of the chest is negative.

Provisional Diagnosis: Tuberculosis? Hodgkin's disease? Sarcoma?

Operation: July 8, 1925. In the left supraclavicular space was a mass the size of an orange. Transverse incision made over the mass. The sternomastoid was retracted medially. The mass was found adherent to the surrounding structures including the internal jugular vein. It was carefully dissected away and removed. The mass had the appearance of malignancy. The operative diagnosis was (?) of malignant lymphoma of the left neck.

Pathologist's Report: DS–25–1039. The specimen consists of a large mass about 9 cm. in diameter. It is rounded and rather coarsely lobulated on the exterior. The cut surface reveals the presence of a coarse fibrous trabeculation, between which is a more or less dark, mushy material which appears somewhat granular, is reddish gray in color and extremely friable. There is a suggestion of a papilliferous type of lesion with extensive secondary hemorrhage and cystic degeneration (Figs. 1 and 2).

Microscopic: The specimen appears to be a papillary adenocystoma of thyroid tissue. There are areas where the cells are so closely packed together that the papillary arrangement is somewhat lost, but most of the specimen presents this feature very markedly. Occasional mitotic figures are found and the cells are arranged with a good deal of regularity. There is no definite colloid. The epithelium is nearly all high columnar in type. Some evidence of inflammatory infiltration is found with foci of polymorphonuclear cells. This is a type of tumor which, while not yet histologically definitely malignant, tends to undergo malignant degeneration with subsequent metastasis so that the prognosis should be extremely guarded and the case treated as potentially malignant.

Diagnosis: Papillary cystadenoma (aberrant thyroid).

CASE 2. *Clinical History:* N. E. D. H. 37,550. Miss E. T., aged 20, entered the hospital November 25, 1926, with a mass of glands posterior to the lower end of the right sternomastoid muscle. The family and past history are unessential.

Present Illness: Recently the patient noticed a mass of glands posterior to the lower end of the sternomastoid muscle in the supraclavicular space. These masses were not tender, did not fluctuate in size, and gave no subjective symptoms. No glands could be felt elsewhere in the body. She had lost 20 lbs. in weight in the past two years. Physical examination was negative except for local.

Preoperative Diagnosis: Tuberculosis of cervical lymph nodes.

Operation: November 24, 1926. A gland about the size of a walnut was removed from the lower portion of the sternomastoid. On section this gland closely resembled aberrant thyroid tissue.

Pathologist's Report: DS–26–2331. The specimen consists of an encapsulated tumor mass measuring about 2 cm. in diameter and roughly spherical. It is purplish red in color and is covered with a serosanguinous fluid. The cut surface consists of soft, friable semitranslucent, pink tissue which tears easily and is finely papilliferous.

Microscopic: It presents a papillary epithelial hyperplasia with some tendency to alveolar formation. There is a very fine loose connective tissue stroma which takes no active part in the proliferation. In various portions there are typical alveoli with some tendency to colloid formation. In several places the hyperplastic tissue is attempting to break through the capsule. Mitotic figures are not in evidence. Histologically the specimen is still benign but it may very likely recur (Figs. 3 and 4).

Diagnosis: Papillary cystadenoma (aberrant thyroid).

CASE 3. *Clinical History:* N. E. B. H. 24,149. Dr. F. W., aged 67. Entered the hospital Aug. 4, 1927, with tumor of the right side of the neck; duration 20 years. The family history and past history are unessential.

Present Illness: The patient has had the tumor for 20 years. It has remained relatively constant in size, shape and consistency. It has never been tender or fluctuant, and has not given any subjective symptoms. There has been no tremor, palpitation or pressure. There has been no loss of weight.

Physical Examination: The physical examination was unimportant except for the local, which showed a fairly large tumor mass deep to the lower portion of the right sternomastoid muscle. An X-ray examination previous to entry showed compression of the trachea from both sides opposite the seventh cervical vertebra. The anteroposterior view showed no abnormalities.

Diagnosis: Large adenoma of the right lobe of the thyroid extending into the thorax and deviating the trachea to the left.

Operation: August 6, 1927. Outside the thyroid body, underlying the sternomastoid muscle, there was a large adherent mass containing a yellow mucoid fluid, and measuring 8 by 4 by 5 cm. There was a similar nodule about 2 cm. in diameter in the lower right lobe of the thyroid. They had no connection. There was some suggestion of malignancy.

Pathologist's Report: DS–27–2010. The specimen consists of two portions of thyroid tissue. The smaller portion is about the size of a large marble and has been dissected from the lower right pole of the thyroid. It is firm on palpation and weighs 12 gm. The cut surface shows the tumor to be well encapsulated. The center of the tumor is made up of a yellowish brown material, granular and fri-

able. From the wall of the adenoma areas of proliferating papilliferous tissue are seen. The second portion of the tissue lay 4 cm. away from the thyroid gland, lateral to it and under the sternomastoid muscle. This mass measures 7 by 4 by 2 cm. and weighs 38 gm. It is well encapsulated. The cut surface presents the same picture as that described for the adenoma.

Microscopic: The sections through the lower pole tumor and the aberrant portion of the gland present an identical histological picture of a papillary cystadenoma. Both appear well encapsulated and show no invasion of the capsule. The epithelium is arranged in the form of papillary projections which have a core of rather dense connective tissue stroma. There is some tendency toward alveolar formation and colloid retention. Some cystic degeneration is seen and a rare mitotic figure. There is considerable arteriosclerosis of the smaller blood vessels (Figs. 5 and 6).

Diagnosis: Multiple papillary cystadenoma (aberrant thyroid).

CASE 4. *Clinical History:* N. E. D. H. 41,390. Miss F. K., aged 21, entered the hospital September 14, 1927, with enlarged glands on both sides of the neck of several months duration. The family history and past history are unessential.

Present Illness: About six months ago the patient noticed a chain of enlarged glands on the right side of the neck along each side of the sternomastoid muscle. The glands have gradually increased in size until the patient feels that her head is pushed to the opposite side. Three months ago a similar chain of glands appeared on the left side. These also have been increasing in size. There was no pain or tenderness over the affected area. There has been no loss in weight and no attacks of sore throat. There was no history of tuberculosis.

Physical Examination: The physical examination was negative except for local. Numerous glands were found on each side of the neck, extending from the mastoid region to the clavicle. They were discrete, not indurated and in no way adherent. They were quite characteristic of Hodgkin's disease. No glands were found in the groin or axillae. The spleen was not enlarged and no mesenteric glands were found.

Biopsy: September 15, 1927. A gland about the size of a lime was removed from the supraclavicular region.

Pathologist's Report: DS–27–2345. The gross specimen consists of several lymph glands which are bound together by adipose and connective tissue. They are pinkish white in color and have a semisolid consistency. There are several cysts which range in size from that of a pea to a small marble and contain a blood-tinged fluid. Microscopically the sections present a picture of a papillary cystadenoma. The villus processes are covered with a columnar epithelium which rests on a connective tissue stroma. Many fields show considerable calcification. The colloid material is moderate in

amount, and many alveoli are seen. A few mitotic figures are seen suggesting possible beginning malignant degeneration.

Diagnosis: Papillary cystadenoma of the thyroid (aberrant); (?) malignancy.

On the basis of the pathological findings operative measures were advised and on September 21, 1927, a complete dissection of the right side of the neck was made. Thyroid tissue was found extending into the chest below, and as high as the submaxillary gland above. It was adherent to the sternomastoid muscle and the internal jugular vein. All portions that were grossly involved were removed. A portion of the thyroid was removed for diagnosis. There was no connection between the gland and the aberrant tissue in the neck.

Supplementary Pathological Report: DS–27–2404. The specimen consists of a mass of glands which are matted together with connective tissue. The total weight is 70 gm. They are very red in color and rather firm in consistency. On section they appear to be fairly well encapsulated and present a picture of aberrant thyroid tissue. There are seen small cysts which contain a brownish-colored fluid and old blood. The gross appearance is not that of tuberculosis or of Hodgkin's disease. One of the glands shows some calcification. Two other small pieces of tissue have been submitted for examination; one is a portion of the internal jugular vein and the other is a lymph gland.

Microscopic: The sections microscopically present a typical picture of a papillary adenocarcinoma. The lymph glands show extensive metastases. However, no invasion is seen of the jugular vein. The epithelium is columnar in type and it rests on a connective tissue stroma which in places shows considerable calcification. The same type of lesion is seen in several of the lymph nodes (Figs. 7 and 8).

Diagnosis: Papillary cystadenoma (aberrant thyroid) with metastases to regional lymph nodes.

The patient made an uneventful recovery and was discharged at the end of two weeks to have subsequent X-ray treatments.

Discussion

Little needs to be added to the data which have been submitted. There is a point of some speculative interest, however, which might be called to attention. In a study of the malignant tumors of the

thyroid gland which we are in process of making, the extraordinary similarity between most of the papilliferous adenomata and these tumors of lateral aberrant thyroid tissue has been constantly noted. This is true not only morphologically, but clinically in many respects. It is in this group of tumors that the prognosis is relatively good. They are slowly growing tumors which tend to spread by direct lymphatic extension, first to the regional lymph nodes. The possibility that this entire group of tumors may arise from these same fifth pouch pharyngeal epithelial rests, in the one case representing displaced cell nests in the course of migration; in the other, displaced cell nests in the process of fusion of the lateral and median portions, is one which it is hard to overlook. It is a theory which does not lend itself readily to proof, but the evidence at hand points strongly in such a direction. This by no means excludes the possibility of a parenchymatous origin for certain of these tumors, but it certainly offers an attractive hypothesis for the explanation of the great majority of them. The proof can be found only in the examination of a sufficient number of cases and the finding of a series along the entire migratory course of the epithelial outgrowth from the posterior pharynx to the thyroid itself. In our series, one of the cases (Case 3) suggests such a possibility; two tumors of identical histology; one within the lower pole of the gland, the other 4 cm. removed.

Pathologically, likewise, little can be added beyond a more detailed discussion of the histological findings. The outstanding features may be cited as the distinct adenomatous character of the tumors with the presence of a well developed capsule; the evidence of chronicity and slow development of the tumors with inevitable calcification, hemorrhage, hyalinization of the stroma and cystic degeneration; the uniform papilliferous arrangement of the cells; and their tendency toward lymphatic extension, as contrasted to the group of solid adenomata (fetal type) which invariably invade the blood stream when they become malignant, as Graham [6] has so succinctly pointed out.

For the sake of completeness it may perhaps not be out of place to call attention to the occasional occurrence of aberrant thyroid tissue in certain of the teratoid tumors; notably, of the ovary. A considerable literature has accumulated during the past few years on this group of cases.

Kovacs [7] recently described a rare tumor of the ovary, goiter-like in nature. Symptoms of exophthalmic goiter had developed with the ovarian tumor and subsided with its removal. The structure of the ovarian tumor was that of a colloid goiter and it seemed not only morphologically and chemically, but functionally, true thyroid tissue.

We have seen one case of aberrant thyroid tissue in an ovarian tumor. The patient, Mrs. B. E. S., aged 40, was admitted to the hospital March 13, 1927. The chief complaint was belching of gas and abdominal distention. For the past six months she had noticed irregular and profuse flowing. The physical examination was entirely negative except for a multiple fibroid uterus. Through a median suprapubic incision a large multiple fibroid uterus was removed together with a multilocular teratoid cyst of the right ovary. The left tube and ovary were left behind. Pathologically the endometrium showed a chronic hyperplastic endometritis, while the leiomyomata presented the usual appearance. Microscopically the cystic ovary showed hair follicles, sebaceous glands and a fairly large area of thyroid tissue characterized by acini lined with cuboidal epithelium and containing colloid material. Thyroid tissue is comparatively rarely seen in these tumors. The patient showed no symptoms referable to an abnormal thyroid physiology.

Summary

Four cases of lateral aberrant thyroid gland tumors are presented, including the clinical and pathological findings.

Their probable etiology is discussed. It is believed that these tumors arise in cell masses (the ultimobranchial bodies) which develop from the posterior portion of the pharyngeal complex, the so-called fifth pouch. This is not a true pouch, but a projection backwards and downwards of the posterior portion of the pharynx. These cells in their migration may fail to meet and fuse with the thyroid and give rise subsequently to tumor formation.

Attention is called to the possible relationship of the papilliferous tumor of the thyroid gland itself to these same cell rests after they have become incorporated in the gland.

Emphasis is placed on the importance of diagnosis in these cases because of their potential malignancy, the difficulty of preoperative differential diagnosis, and their relatively favorable prognosis.

Postoperative X-ray treatment is recommended for all cases.

A case of aberrant thyroid tissue in a multilocular teratoid cyst of the ovary is described.

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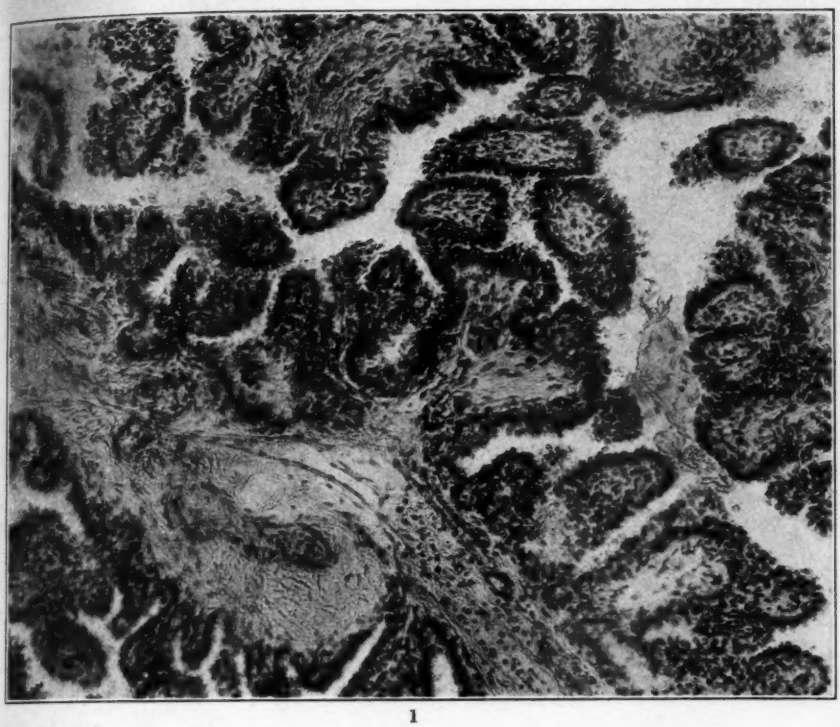
DESCRIPTION OF PLATES

PLATE 107

FIG. 1. Low power photomicrograph. Illustrates the papillary hyperplasia. (Case No. DS–25–1039.)

FIG. 2. Same as Fig. 1, high power, showing the arrangement of the cells on papillary stalks.



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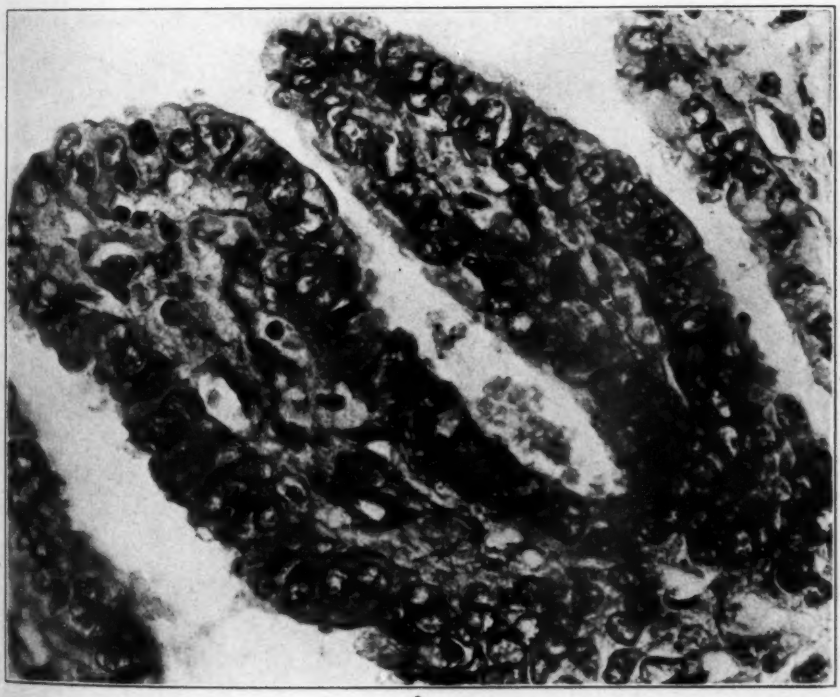

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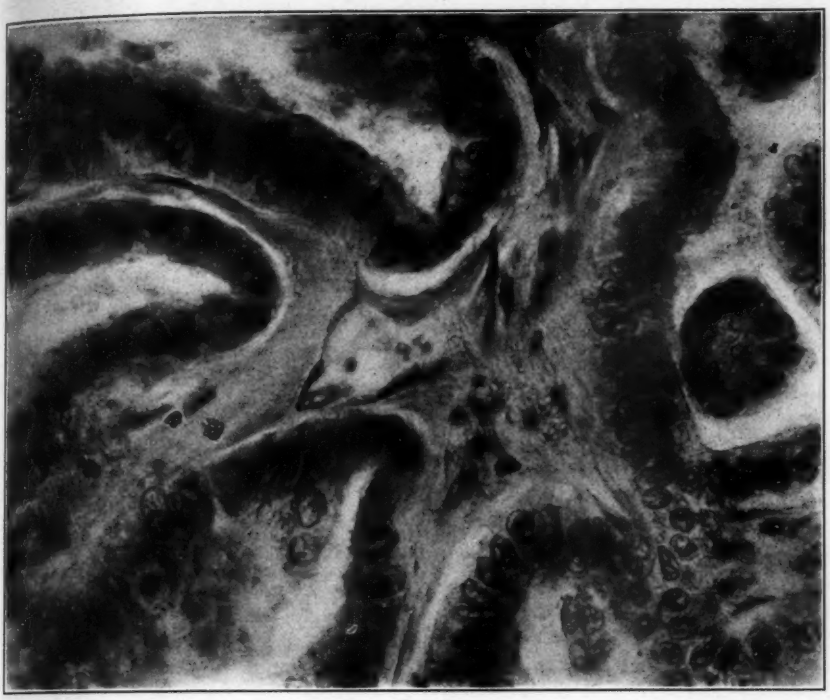
PLATE 108

FIG. 3. Illustrates the relatively benign appearance of these tumors in their earlier stages. Note the marked papillary hyperplasia comparable to that seen in the papillary cyst adenomata of the ovary. (Case No. DS–26–2331.)

FIG. 4. Same as Fig. 3.



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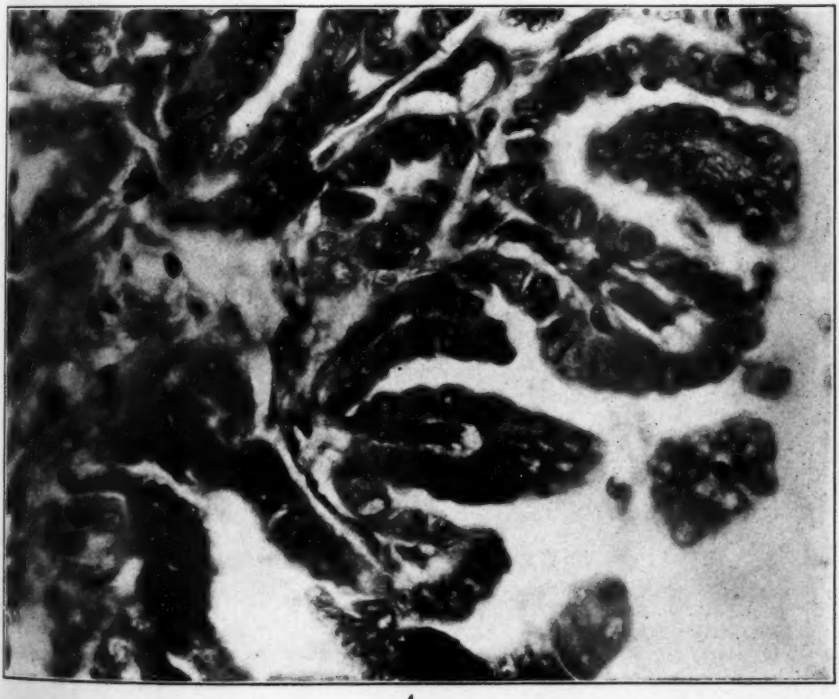

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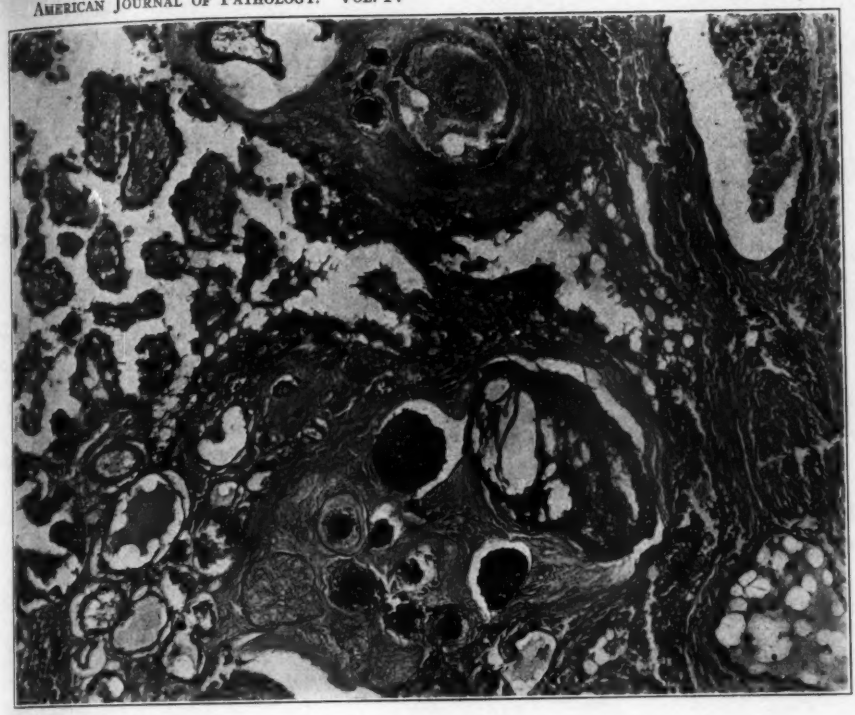
Leech, Smith and Clute Aberrant Thyroid Glands

PLATE 109

FIG. 5. Low power photomicrograph of an area through the wall of the tumor. This emphasizes the long duration of certain of these cases with marked fatty degeneration and calcification. (Case No. DS–27–2010.)

FIG. 6. High power, same as Fig. 5.



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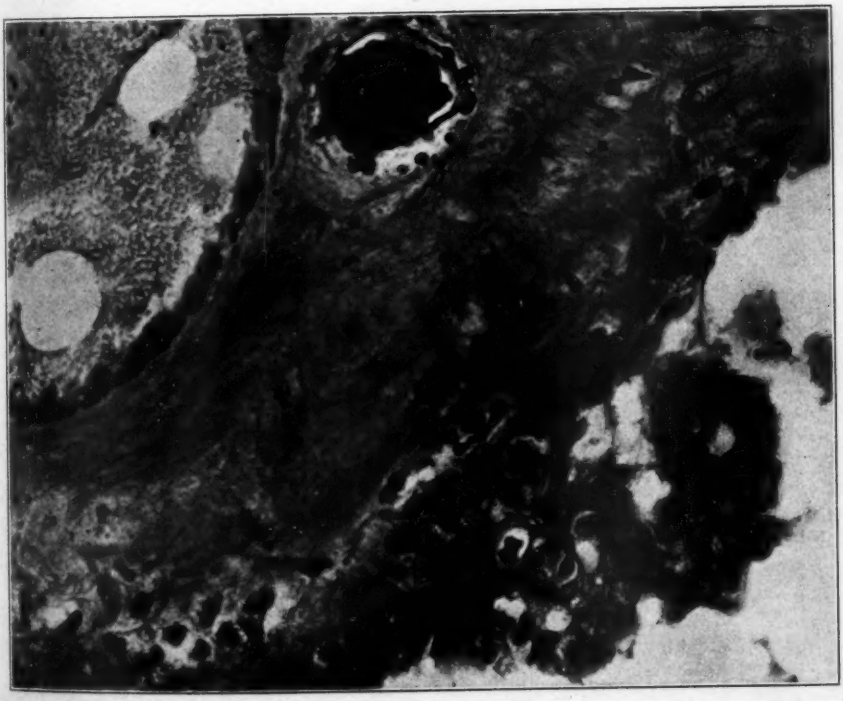

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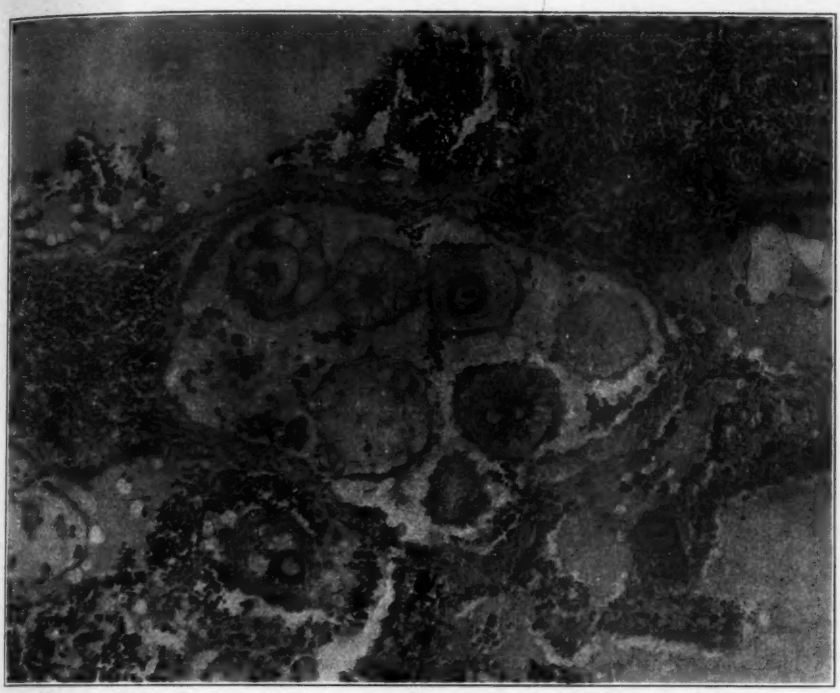
PLATE 110

FIG. 7. Low power photomicrograph illustrating the invasion of the regional lymph nodes of the neck by tumor tissue. The slow rate of growth is emphasized by the papillary proliferation, cystic degeneration and absence of mitotic figures. (Case No. DS–27–2345.)

FIG. 8. High power, same as Fig. 7.



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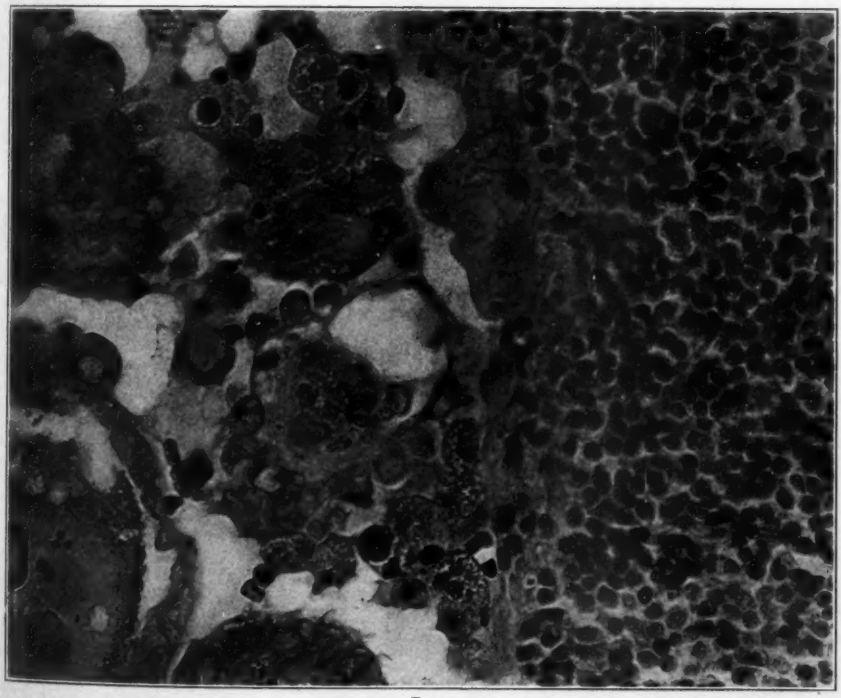

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STAINING FIBRILLARY NEUROGLIA IN FORMALIN-FIXED MATERIAL *

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Mallory's [1] excellent phosphotungstic acid hematoxylin method for neuroglia is applicable only to Zenker-fixed material. Bailey's [2] more selective stain for fibrillary neuroglia with neutral ethyl violet-orange G was also developed specifically for material preserved in this fixative. This leaves for formalin-fixed material practically only the method of Weigert which, in addition to being an involved procedure and requiring expensive chemicals that are often difficult to obtain, gives blurred and indifferent pictures on material fixed in formalin for long periods before mordanting.

I have tried refixing formalin tissue in Zenker's solution but the results on such material with the methods either of Mallory or of Bailey are not worth the effort.

For many years Cajal and Del Rio Hortega have advised the use of ammonia for removing an excess of formalin from sections for various silver stains. Recently Globus [3] has applied this principle to the staining of material fixed in formalin alone with the Spanish silver methods which originally required fixation in formalin and ammonium bromide. He treats frozen sections cut from formalin-fixed blocks with ammonia overnight in a warm oven and then "bromurates" them with hydrobromic acid. Upon sections thus treated he applies the various silver techniques with excellent results.

This being the case, I have treated formalin-fixed blocks with ammonia to remove the formalin, refixed them in Zenker's solution and stained sections cut from paraffin blocks by the methods of Mallory and of Bailey in order to stain the neuroglia fibrillae. The procedure is as follows:

(1) Blocks of tissue 2 or 3 mm. thick are cut from the formalin-fixed brain, cord, or tumor, and placed in a dish filled with about 100 cc. of distilled water containing 30 to 40 drops of strong ammonia water. This is kept air-tight in an oven at 37° C for 4 days.

(2) The blocks are washed for 12 to 24 hours in running water.

(3) They are then fixed in Zenker's solution for 24 hours.

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(4) Embedding in paraffin, cutting and staining are then carried out as described by Mallory or Bailey, to whose work the reader is referred.

The results obtained by this procedure are exceedingly satisfactory. Within limits of fifteen to twenty years, the length of time that material has been fixed in formalin does not seem to affect the quality of the stain. The phosphotungstic acid hematoxylin stain brings out the cytoplasm of the protoplasmic neuroglia as well as the fibrillary neuroglia and fibrillae, whereas the ethyl violet-orange G method stains the fibrillae more specifically as well as the fibrillary astrocytes. The latter stain, moreover, does not stain myelin sheaths or connective tissue as the Mallory procedure is likely to do.

Unfortunately the difficulty in preparing the neutral ethyl violet-orange G stain has prevented its general use. This difficulty, however, has been completely overcome by Dr. Bailey, who is permitting me to publish his present simple method of preparation of his stain, which is as follows:

(1) Mix ethyl violet 1.0 gm. } accurately
Orange-G 0.5 } weighed
(2) Add 100 cc. distilled water and stir thoroughly.
(3) Place in or on a warm oven (37° C) for 12 to 24 hours to precipitate.
(4) Decant supernatant fluid and wash precipitate several times with distilled water.
(5) Place in oven to dry.
(6) Make a saturated solution of dried precipitate in absolute alcohol.

This solution, if well stoppered, will keep indefinitely.

For staining, use one part stock solution to three parts of 20 per cent alcohol.

One difficulty may arise from the fact that not enough material is on hand at times to permit the assignment of a whole block for this purpose. In fact, it may happen that all the tissue available consists of a single formalin-fixed block which is already embedded in paraffin. In this case, too, it is possible to obtain a very satisfactory refixation as follows:

(1) Sections * are deparaffinated and placed in 50 cc. of water containing 10 drops of ammonia for 24 hours at room temperature.

* Sections to be treated with strong alkalis are less likely to float off the slide if, instead of egg-albumin and glycerin, gelatin is used to make them stick on. For this purpose 2 square inches of pure gelatin are dissolved in 250 cc. of boiling water. The sections are floated on the surface of this solution while still quite warm, and drawn up on clean slides. The excess moisture is wiped off and the slides put for 24 hours into a warm oven (37° C) in which a beaker of formalin stands open.

(2) The ammonia is washed out with a slow stream of running water for 1 to 2 hours.
(3) Sections are refixed in Zenker's solution for 12 to 24 hours,
(4) Washed in running water 3 to 4 hours,
(5) Treated with iodine solution to remove the bichloride crystals and stained.

The resulting stain is not so deep or so sharp as when the tissue is treated in block before embedding, but is still remarkably fine. It is not unlikely that equally good results as from refixation in block could be obtained if the sections were better ammoniated, but such a procedure only serves to float off the section from the slide and ruins the preparation.

Comment: By this method, it is possible to obtain excellent preparations to show neuroglia fibrillae in material which, owing to its long fixation in formalin, does not yield itself to the satisfactory demonstration of these elements by any other means. The importance of this is evident when it is recalled that for numerous decades formalin fixation, especially for the central nervous system, has been not only the favorite, but, in many instances, the sole preservative employed.

In dealing particularly with the gliomatous tumors of the nervous system where a reliable and specific method for the demonstration of glia fibrillae is often essential for their classification, this method may serve to save much valuable material which might otherwise be wholly useless, or at least unsuited for thorough investigation in the light of recent developments in our knowledge of these neoplasms.

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DESCRIPTION OF PLATE

PLATE III

FIG. 1. Astrocyte from fibrillary astrocytoma. Phosphotungstic acid hematoxylin. Stain carried out upon formalin-fixed material after treatment by ammonia and refixation in Zenker's solution. × 1000.

FIG. 2. Neuroglia fibrillae from a brain showing cortical sclerosis. Ethyl violet-orange G. Formalin-fixed material; ammonia treatment; Zenker refixation. × 1000.

FIG. 3. Neuroglia fibrillae from the same case as Fig. 2. Ethyl violet-orange G. Treated in the same manner as the above. × 250.

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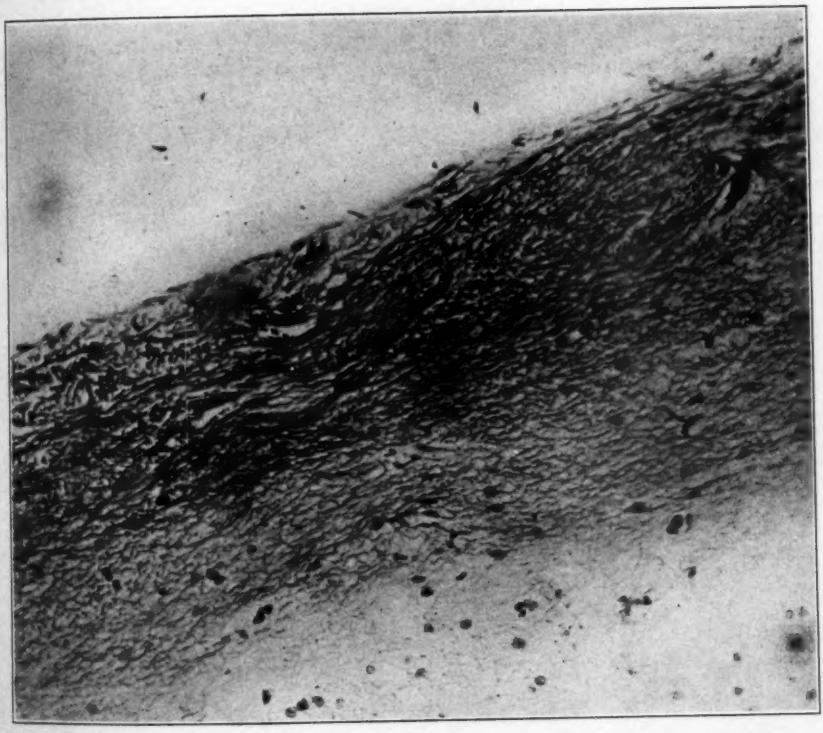
2



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Davidoff

Staining Fibrillary Neuroglia

MULTIPLE PRIMARY NEOPLASMS IN LOWER ANIMALS *

REPORT OF A CASE

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To the pathologist interested in the study of human tissue the occurrence of two or more primary heterogeneous neoplasms in the same person is not an uncommon observation. On the other hand, to the student of comparative pathology who is privileged to see a relatively small number of tumors in animals the finding of multiple primary neoplasms in an animal is likely to be considered a pathologic event, at least worthy of a brief description. I do not mean to infer that the lower animals are less prone to neoplastic proliferation than man or that in them there are fewer multiple tumors. The fact is that if the lower animals were permitted to live out their natural span of life most of them might show a tumor incidence comparable to that of man.

The apparent discrepancy in the incidence of neoplasms in man and lower animals is due to several factors, the most important of which are: (1) the comparatively early age at which the majority of animals, particularly the meat-producing animals, die, and (2) the failure to make a thorough necropsy examination on animals that die from natural causes. Even many veterinarians, whose training should have incited at least ordinary interest in morbid pathology, will not perform a necropsy unless especially urged to do so and necropsies by lay owners are not of any value in the acquisition of pathologic data. As a result only a small percentage of animals dying from natural causes are carefully examined by someone directly interested in the pathologic anatomy of disease.

The finding of more than one primary tumor in the same individual has always resulted in renewed interest in the subject; thus, many cases have been recorded in the literature. This is particularly

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true in cases occurring in human beings. Although foreign literature contains reports of many cases of multiple neoplasms in lower animals the cases reported in English literature are rare.

In this brief review an attempt has not been made to present a complete summary of the literature. Enough cases have been reviewed, however, to enable one to grasp a more comprehensive conception of multiple primary neoplasms as they occur in animals than would be possible from the one case reported herein.

Multiple tumors of the thyroid gland in a dog were described by Schöne.[1] In the thyroid gland was a typical primary carcinoma with extensive metastasis to the lung, and a spindle-cell sarcoma which failed to metastasize. Schöne believed that the sarcoma resulted from degenerating epithelial elements, or was at least secondary, and related to the carcinoma.

Betke [2] reported multiple primary neoplasms in a forty-year-old captive rhinoceros. In the uterus was a fibromyoma and in the mucosa of the cervix were two large papillary adenocarcinomas. Metastasis, even to the regional lymph nodes, was not demonstrated.

Wooldridge [3] noted adenoma of the prostate and adenocarcinoma of the liver in one case. The animal was a fourteen-year-old fox terrier. The prostate contained a mass weighing 1 kg. In spite of the huge size of this tumor, normal micturition was possible. Multiple adenocarcinomas of the liver were also found.

Ball [4] found in a nine-year-old poodle an epithelioma in the pancreas and a sarcoma of the intestine situated close to the pylorus. Metastasis to the lungs, spleen, and kidney was also noted.

Mettam [5] recorded the case of a seventeen-year-old pointer with a recurrent adenocarcinoma in the anal region, metastasis to the pleura of the right lung and spindle cell sarcoma in the mesentery near the ileocecal juncture. The intestine was not involved. On the surface of the spleen were three tumor-like nodules.

Bartlett [6] described multiple tumors in two dogs. In one there was adenocarcinoma of the thyroid gland with metastasis to the lung, mediastinum and pineal region, bilateral mesotheliomas of the suprarenal glands, and cavernous hemangioma of the liver; in the other, adenocarcinoma of the thyroid gland with metastasis to the lungs, a chondrosarcoendothelioma of the mammary gland and multiple adenomas of the suprarenal capsule.

Boyd, Fitch, Grinnells and Billings [7] observed multiple adenomas

of the pancreas and a cavernous hemangioma of the liver in a ten-year-old Holstein cow.

Fox [8] in an extensive experience with captive wild mammals and fowls recorded only one case, that of a jaguar (*Felis onca*), in which multiple primary neoplasms were encountered. The tumors were as follows: fibro-adenoma of the uterus, fibro-adenoma of the bile ducts, and lymphangioma of the mesentery.

Houdemer and Bablet [9] described the case of a dog in which lymphoblastoma of the inguinal lymph nodes and papillomas in the penis were found.

In a paper dealing with old age in relation to cell overgrowth, Goodpasture and Wislocki [10] recorded the finding of a total of ninety tumors in an examination of fifteen old dogs. The tumors in the respective animals varied from three to eleven. There might be some hesitation in accepting the entire ninety tumors as true neoplasms; nevertheless, the paper emphasizes in a forcible manner the tendency of tissues (at least in dogs) to advancing proliferation with the advent of senescence. In a later paper Goodpasture [11] discussed exhaustively the relation of the age of dogs to tumors and presented a summary of his observations in another series of thirty-five old dogs. In the fifty dogs studied 228 tumors were found, thirteen of which were classed as malignant and 215 as benign.

Perhaps the most comprehensive paper in recent literature dealing with multiple neoplasms in the lower animals is that of Cohrs [12] who, besides presenting an extensive bibliography, recorded his own valuable observations. In a total of 737 necropsies on dogs he found tumors in seventy; in twenty-six of these the tumors were multiple primary neoplasms. He described one case in particular in which there was a most unusual collection of neoplastic and related anomalies. The animal was a male dachshund aged 14 years. Clinical examination failed to reveal anything of diagnostic significance aside from polyuria and excessive thirst and the dog remained in good flesh until death. At necropsy a veritable museum of abnormalities was found: melanoma of the skin with beginning spindle cell sarcoma, multiple hard papillomas of the skin, ulcerated squamous cell carcinoma of the skin low in the median line, multiple sebaceous adenomas of the skin, multiple adenomas of the anal gland, papillary adenoma of the lungs, cortical malignant hypernephroma of the left suprarenal gland, cortical malignant hypernephroma of the right

suprarenal gland, primary carcinoma simplex and adenoma of the right testis, multiple carcinoma simplex of the left testis, nodular hyperplasia of the pancreas, and areas of nodular hyperplasia in the spleen.

A review of the papers of Goodpasture and Cohrs makes me somewhat hesitant to present the one case it was my privilege to study. I offer it, however, as a matter of record, not with the assumption that the observation of one case is important, so far as the conception of multiple neoplasms is concerned.

Report of Case

Clinical History: The dog was a thirteen-year-old male shepherd. From the time he was a puppy he had been fondly regarded as a member of the household and for a considerable portion of his life his habits had been sedentary. About two years before he died squamous cell carcinoma, graded 3, developed from the mucosa of the gum of the mandible in the region of the incisor teeth. This was removed surgically and healing ensued. About a year later recurrence was noted. The tumor continued to grow and to encroach on the soft tissues. The incisors were overgrown by cancerous tissue and the canines were loosened and pushed out of their normal position (Fig. 1). The lips were not affected. Debility increased gradually and for humane reasons the owner asked to have him killed.

Necropsy: Anomalies were as follows: a smooth, flattened, irregular tumor on the anterior floor of the mouth, the tumor encroached on the lower incisor teeth, which were loosened as were the canines; enlarged left submaxillary lymph node; enlarged thyroid gland, weighing 35 gm.; a large cyst 6 cm. in diameter in the liver near the periphery with many smaller pale areas just under the capsule, from 0.2 to 1 cm. in diameter, the surface of these areas being slightly depressed; large multiple nodules over the surface of the spleen; a grayish white irregularly spherical tumor measuring 2 cm. in diameter firmly adherent to the wall of cecum; prostate gland greatly enlarged, weighing 160 gm., and a yellowish mass irregularly spherical and measuring 1.8 cm. in diameter in the right testis (Fig. 2).

Pathologic Anatomy: Suitable material was selected from the various lesions and prepared for microscopic study.

The tumor of the mouth was a typical squamous cell carcinoma, graded 3 (Fig. 3), and similar in every respect to the primary tumor which had been removed two years previously. The tumor was covered by a thin layer of mucosa and near the surface were a few

areas of necrosis with infection. The trend of the growth was downward into the surrounding tissues rather than outward.

The left submaxillary lymph node possessed but few landmarks by which it could be identified as a lymph node. Large areas had undergone necrosis with dissolution of tissue, and the lymphoid cells were limited to a few isolated clumps near the periphery at one side. The bulk of the structure consisted of carcinomatous cells and a considerable amount of fibrous connective tissue. Many of the carcinoma cells were much altered in appearance and showed a decided tendency to assume an elongated rather than an oval contour. The influence exerted by the advancing fibrous tissue was apparent, although in spite of this inhibition the carcinoma cells retained a certain amount of aggressiveness and mitosis was easily demonstrated. Although the tumor cells show some alteration due to the associated connective tissue elements there can be no reasonable doubt that the tumor of the lymph node represents metastasis from the primary tumor in the mouth. There were many melanotic granules in what remained of the lymph tissue.

Sections of the liver were obtained through the pale depressed areas and these were found to consist of irregularly branching cavernous channels lined with a flattened type of cell which was apparently endothelial in nature (Fig. 4). The interior of many of the channels was occupied by variable quantities of red blood cells. The hepatic tissue in the tumorous areas was entirely obliterated. In some areas the channels were pushing into the adjacent substance which was in a state of congestion in the zone immediately surrounding the tumorous area. There seems to be sufficient evidence to consider this anomaly, hemangioma of multicentric origin.

The tumor of the cecum was a highly cellular, rapidly growing type of neoplasm which occupied a position in the wall of the intestine, extending from the serosa to the muscularis mucosae. In the tumorous area no vestige of intestinal musculature remained and the tumor was clearly encroaching on the adjacent muscle tissue. Even the muscularis mucosae was invaded in some instances by the advancing tumor cells although the mucosa was not disturbed. The cell was typical of smooth muscle tumor; the anomaly was designated malignant leiomyoma (Fig. 5).

Sections of the spleen were obtained from several of the splenic nodules. While the gross lesions were striking, the microscopic

picture was somewhat disappointing and decidedly difficult to interpret with any degree of confidence. The nodules consisted of extensive areas of red blood cells with a variable number of large irregular lymphoid cells, some of which appeared decidedly embryonic. The condition was considered multiple nodular lymphomatous hyperplasia.

The thyroid gland had suffered profound alteration and few elements remained which were common to the normal thyroid gland. Most of the alveolar spaces were filled with red blood corpuscles, and a meager quantity of colloid substance was present in a few of the remaining alveoli. The most striking change, however, was the tremendous increase in the interstitial connective tissue of the gland. This was so profuse in some instances as to cause a fusion of large areas with resultant hyalinization. As a consequence many of the alveoli were obliterated entirely. The diagnosis was chronic fibrous thyroiditis.

Normal prostate tissue was not found in any of the sections of the prostate gland examined. The structure was that of multiple cystic cavities lined with high columnar epithelial cells, the nucleus of which was situated near the attached end. While the epithelium lined many of the cysts in a smooth and orderly fashion, the reverse was true in the majority of the fields in which the epithelium was projected into the interior of the cysts from all sides in great profusion (Fig. 6). The rugae or papillae were of variable height and the high columnar type of epithelium was maintained throughout. A colorless, lace-like residue was present in several of the cysts. Mitosis was not observed in any of the cells. The amount of fibrous tissue was not increased and the epithelial cells did not show a tendency to invade the surrounding stroma. A diagnosis was made of papillary cystadenoma.

The tumor of the testis was sharply demarcated from the surrounding testicular elements by a zone of fibrous connective tissue and while the adjacent seminiferous tubules were clearly undergoing a retrogressive change they were in no immediate danger of being replaced by the neoplastic cells. The tumor cells were rather large, polyhedral, and closely packed together. Delicate strands of fibrous tissue separated small groups in rather indistinct units (Fig. 7). The cellular cytoplasm was inclined to stain lightly although the nuclei stained well. A nucleolus was observed in most of the cells, and

most of them had a rather high lipoid content. Mitosis although present was observed infrequently. A diagnosis was made of interstitial cytoma of the testis.

Briefly summarized, the following morbid changes were observed: squamous cell carcinoma of the mouth with metastasis to the submaxillary lymph node; multiple hemangiomas of the liver; malignant leiomyoma of the cecum; papillary cystadenoma of the prostate and tumor of the interstitial cells of the testis; multiple lymphomatous nodules of the spleen, and fibrous thyroiditis.

Comment

The anomalies reported in this case represent five true tumors with splenic changes, perhaps a related neoplastic expression.

Considering the relative frequency of tumors of the thyroid gland in the dog it is somewhat surprising that the thyroid gland in this case should reveal chronic inflammatory fibrosis rather than a neoplastic tendency. Certainly the general condition of the animal was favorable although a certain amount of local inhibition must have existed, or all the tissues would have presented neoplastic changes. If the animal had been permitted to live until life was terminated as a consequence of the influence of the neoplasms or as a result of other senile delinquencies, it is probable that other tumors would have become demonstrable.

In considering the influences underlying the appearance of several primary tumors in the same individual, age seems to have much significance. This has been commented on many times by other observers. Goodpasture and Wislocki who based their opinion of age in dogs they studied by the presence of the usual senile changes, such as loss of teeth, the presence of cataract and the general decrepit condition, said: "In no animal with extensive wasting and loss of teeth have we failed to find tumors in more than one organ." Goodpasture remarked that the fifty dogs constituting the basis of his study were chosen because of evidence of advancing age and in no instance was a dog presented for study because of the presence of a tumor. Smith[12] studied the senile changes of the testis and prostate in dogs; he used thirty-two animals varying in age from six weeks to twenty years. The fifteen animals that presented evidence of being ten years old or more than ten showed definite tumors at

necropsy. As regards the possible relationship of advanced age with the appearance of neoplasms in dogs, the observations of Cohrs are significant. Of the twenty-six animals in his study who had two or more primary tumors all but one were more than ten years of age.

The part that age plays in the occurrence of tumors, especially in dogs, is further emphasized by the relative infrequency with which neoplasms are seen in young animals. In a series of several hundred necropsies conducted during the last year very few tumors were found in dogs less than five years; most of them were found in the older animals.

It would seem that the explanation of the greater frequency of both single and multiple tumors in the older animals is definitely related to and dependent on senile involution of the respective tissues. Goodpasture said: "With age there is a progressive differentiation which eventually injures the cells of the body. Many of the cells die, others become dedifferentiated in varying degrees. The dedifferentiated cells possess the power to grow; but their capacity to function may be diminished or lost. From these dedifferentiated cells metaplasia and benign and malignant tumors arise."

The above hypothesis suggests the possibility that every tissue of the body may give rise to neoplastic overgrowth and ignores, or perhaps underestimates, the possible influence of heredity in the occurrence of tumors in the aged. It would be extremely important to know whether a dog with an established cancer-resistant ancestry which would protect it during the first ten years of life would develop single or multiple tumors due to a predominating influence exerted by the forces underlying the changes of senile involution.

The frequent occurrence of neoplasms in the older dogs makes it difficult to accredit heredity as providing the dog with any considerable amount of protection from tumors during the period of senile retrogression. On the other hand, it is difficult to believe that most dogs of advanced age possess a dormant hereditary susceptibility for neoplasms. Certainly there is a problem in genetics presented here which makes it impossible to know definitely what part, if any, heredity plays in the inception of lawless overgrowths of tissue in old dogs. Goodpasture's hypothesis that cells which have suffered injury due to age and have in consequence experienced loss of specificity and assumed an increased power to grow, appears insufficient to explain the entire phenomenon.

In the case of dogs neither sex nor breed appears to bear any relation to the incidence of multiple neoplasms.

Summary

From a brief review of the literature it is apparent that primary, multiple neoplasms may appear in a great variety of species. Multiple neoplasms are frequently noted in necropsies on dogs more than ten years of age. This suggests the influence of senile retrogression and subsequent tissue involution in the etiology of these tumors. The possible influence of heredity is a factor which warrants investigation. A case of multiple primary neoplasms in a thirteen-year-old dog is presented. The tumors described are: recurrent squamous cell carcinoma of the mouth with metastasis to the regional lymph node; multiple hemangiomas of the liver; malignant leiomyoma of the cecum; papillary cystadenoma of the prostate, and a testicular tumor arising from the interstitial or Leydig cells. Multiple lymphphomatous nodules of the spleen are also present.

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DESCRIPTION OF PLATES

PLATE 112

FIG. 1. Primary carcinoma of the anterior floor of the mouth. The cancerous tissue has caused the teeth to be pushed from their normal position.

FIG. 2. Interstitial cytoma of testes. Tumor is sharply demarcated.

FIG. 3. Primary squamous cell carcinoma of the mouth. One cell in mitosis. × 1350.

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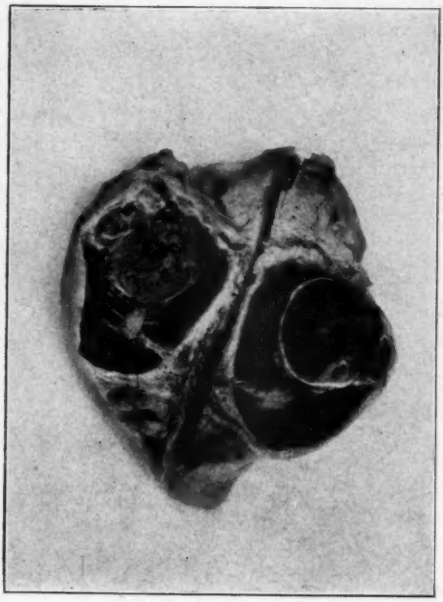

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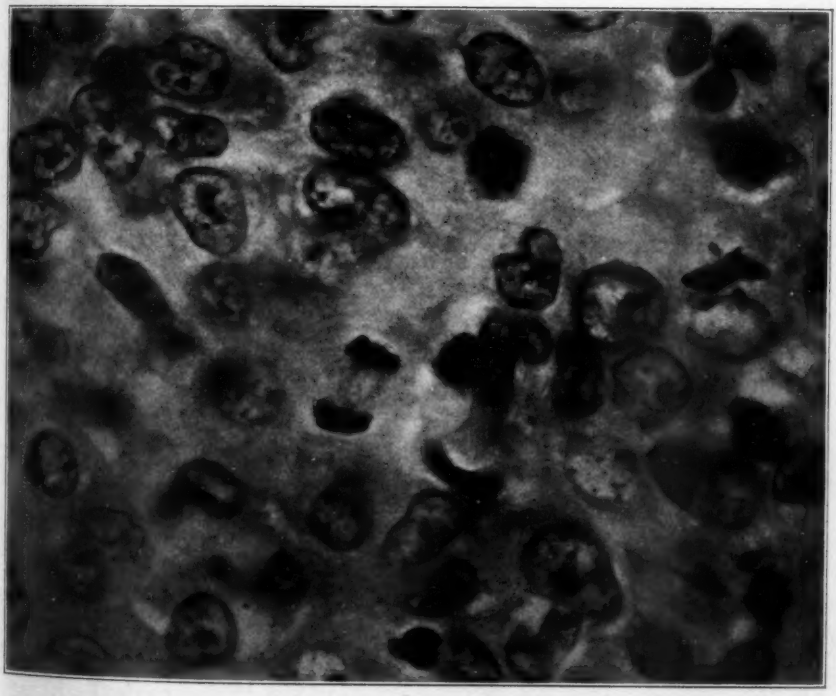

3

Feldman

Multiple Primary Neoplasms in Lower Animals

PLATE 113

FIG. 4. Hemangioma of the liver. Tortuous channels occupied by variable quantities of erythrocytes. × 50.

FIG. 5. Leiomyosarcoma of the cecum. One mitotic figure present. × 1350.

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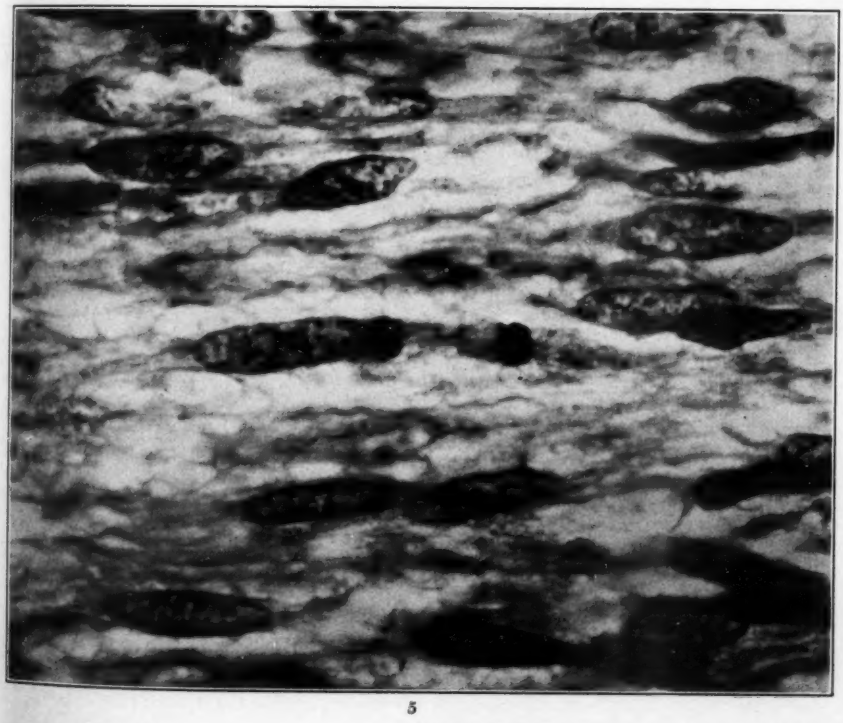

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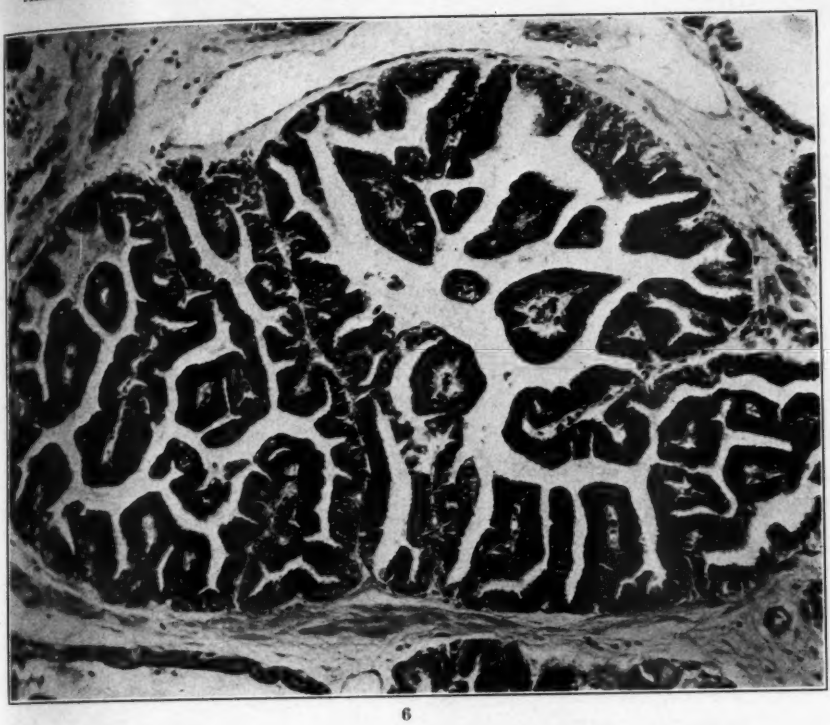
Feldman

Multiple Primary Neoplasms in Lower Animals

PLATE 114

FIG. 6. Papillary cystadenoma of the prostate gland. A richly cellular area showing the extensive overgrowths of cells, the majority of which assume a papillary type of arrangement. × 150.

FIG. 7. Interstitial cytoma of the testis. Compact arrangement of the neoplastic cells; clear, somewhat granular cytoplasm and prominent nuclei. × 770.



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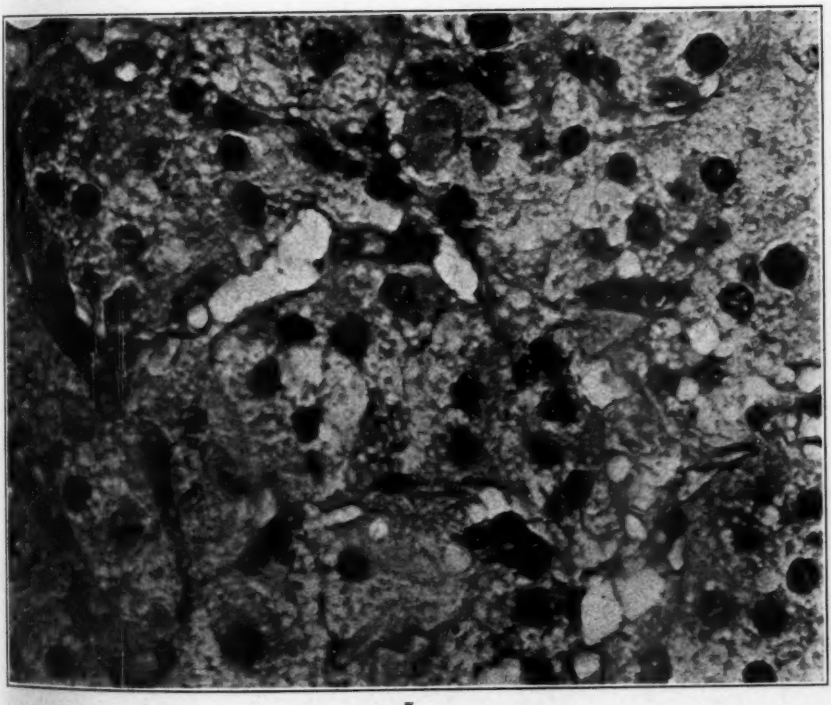

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Multiple Primary Neoplasms in Lower Animals